The Reproductive Ecology and Biology of the Pill-box Crab: *Halicarcinus cookii* (Brachyura: Hymenosomatidae) Filhol, 1885

A thesis submitted in partial fulfilment of the
requirements for the Degree of

Master of Science in Zoology

in the School of Biological Sciences
at the University of Canterbury

by

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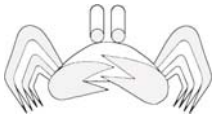
University of Canterbury

2006



This study investigates the reproductive strategies of the pill-box crab, *Halicarcinus cookii* on the Kaikoura Peninsula, New Zealand. Various aspects essential to understanding reproductive strategies were examined including growth, population dynamics, reproductive biology and mating behaviour. *H. cookii* exhibits obvious sexual dimorphism such that females develop wide abdomens forming brood chambers, and males tend to grow larger than females and have larger chelipeds in relation to body size. *H. cookii* allocates energy into growth and reproduction in separate phases of its life cycle where growth ceases as reproductive maturity begins due to a terminal/pubertal moult. Despite the presence of ovigerous females throughout the 15 month sampling period, the population was highly seasonal, with peaks in recruitment and growth occurring primarily during the winter months and peaks in numbers of mature individuals during the summer months.

Reproductive output increased with body size in *H. cookii*, as larger females produced more eggs and larger males transferred more sperm than their smaller counterparts. Ovaries matured prior to the terminal/pubertal moult (anecdysis) and, in multiparous females, in synchrony with brood development, allowing females to produce broods in quick succession, maximising their reproductive output in their short life span (approximately 12-18 months, 6 months as an adult). Incubation duration of broods decreased as seawater temperature increased, suggesting that temperature is the primary cause of the seasonal population cycling.

Sperm storage allowed females to produce at least 4 fertilised broods without re-mating. Some sperm mixing in the spermathecae appeared to occur and the ventral-type structure implies last male sperm precedence. Males therefore preferentially mated with females closest to laying a new brood and guarded them longer than other females to ensure their paternity. Guarding duration varied according to the sex ratio allowing males to maximise their reproductive output.

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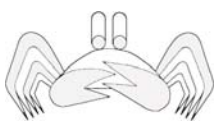

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Acknowledgements

So many people, so little space! First thanks to **God**, for creating all the amazing wildlife in the world for us to study and the intelligence in (some of) us to study it. Thanks also to **my parents** for their financial, emotional, spiritual and gametal input into my life, without them, I would not exist. Big thanks to the rest of **my family** also for their encouragement and support, especially to **Jonathan** and **Hannah van den Brink** for providing the beautiful crab drawings. Also thanks to my closest friends **Leah Simpson (Taylor)**, **Amanda Bradley** and **Elizabeth Williams (Bryant)** for sharing with me the experiences of the educational institution since we were 11 years old, BBQ kettlefries, muesli bars, dances, chick flicks and endless girly chats, I love you guys!

Particular thanks to my supervisor **Colin McLay** for all the help and advice in planning, producing and proof reading during the last two years, **Mathis Hasler** for his sterilizing help at the Christchurch Oncology Department, **Neil Andrews** for helping with all the SEM stuff and **Jan McKenzie** for her eagerness to help out with the many blown bulbs- I still don't know how that light source got full of dead flies! I blame Hosie.

Thanks to the **MERG** crew, for adopting a poor, lonesome crab researcher as one of their own, at least when it came to the fun stuff, and the **FERG** bunch for allowing me to share the office in Chch, bug polo and their laser strike team against MERG.

Thanks to **Jack** (box of birds) **van Berkel** for the accommodation, crappy coffee, endless anecdotes and amusing stories about Kaikoura's sordid past, to **Wayne Nelson** for all the techniciany stuff without which the lab could not operate, and **Hine** and **Ngarie** for their meticulous cleaning services. To all the Kaikoura lab rats over the years: **Katie** (suck it up and deal) **Lotterhos, Stacie** (just shut up! I have something to say: I love you guys!!) **Lilley, Robyn** (insane athlete woman) **Dunmore, Sheba** (Grrr RUF RUF) **Mongrel, Warrick** (Spelt is one of the worlds oldest grains) **Lyon, Laura** (super seal woman) **Boren** and **Mark** (save the whelks) **Novak**, thanks for putting up with me. Also thanks to **Andrew** (puppies…box full of puppies) **Hosie** for being more the 'support and mentor' than he had to be...

To the others in Kaiks, many thanks, particularly to the lovely pilots **Dean** (I'm taller than you) **Strang** and **Matt** (I had a crush on MacGuyver) **Kerr** who was also a lovely flatmate (I can still kick your ass though!), to **Sara** (hurt my ankle again) **Mulhearn**, a lovely flatmate, exercise guru and horse riding buddy, **The Olive Branch** for funding me to buy coffees wine and food, **The Sonic**, **Adelphi**, **Dolphin Encounter** and **Hislops** for selling me coffees wine and food and **Sonic Mojo** and **Good Acting Gary** for entertaining me while buying coffees wine and food (well…mostly wine), and to the girls who made winter 2005 the most fun ever with dress up parties, biking pub crawls and all the random adventures: **Sarah, Paula, Livi, Celia and Jocelyne** (WOO YEAH!!).

Finally a huge thank you and big hugs to my partner in crime, room sharing buddy (both in the field station and at the flat -I still can't believe we haven't killed each other), confidant and all out maker of mischief...**Kimberley Seaward**. Without you I would not have survived Kaikoura, let alone university.

"I have never lost this fascination, or the sense of mystery, or the sense of awe at the creator of this world. I am a rational scientist -- but also a fascinated child."
- Ass. Prof. David Given 1943-2005

Crab Muffins

INGREDIENTS:

- 1 pound crab meat, cooked and drained (reserve juice)
- 1/2 cup finely chopped onion
- 1/2 cup finely chopped celery
- 2 eggs, beaten
- 1 teaspoon prepared mustard
- 1 teaspoon Worcestershire sauce
- 8 to 10 slices white bread, crumbled
- 1 tablespoon butter

PREPARATION:

In a skillet over medium heat melt the butter; stir in the onion and celery. Sauté for about 4 minutes, or until the onion is translucent.
In a large bowl mix crab, beaten eggs, celery and onion mixture, Worcestershire sauce and mustard. Gently stir in the bread crumbs a little at a time, until the mixture resembles a moist stuffing. If too dry, add some of the reserved juice from crab meat. Grease muffin pan with cooking oil spray. Fill each muffin cup two-thirds full. Bake at 180°C for 45 minutes or until an inserted toothpick comes out clean. The crab muffins freeze well for about 6 months without freezer burn.

Chapter 1

General Introduction

Sexual selection is a major driving force in the evolution of a species. Due to the country's isolation and corresponding unique selection pressures, New Zealand is rich in its variety of endemic species whose life histories are relatively unknown at this time. The aim of this thesis is to investigate the environmental, biological and behavioural components of the reproductive strategies of the endemic pill-box crab *Halicarcinus cookii* around the Kaikoura Peninsula, New Zealand. Although studies of similar hymenosomatid species have been conducted in the area already (Dunnington, 1999; Hosie, 2004; Menzies, 1988), *H. cookii* has never been studied as an individual species. The research presented in this thesis will provide another piece to the puzzle of the unusual reproductive patterns characteristic of many hymenosomatids (Lucas, 1980).

1.1 Background

Competition over mates takes many forms and has consequences that can be a driving force in sexual selection (Andersson and Iwasa, 1996). Sexual selection involves the selection for biological traits resulting from intra-specific competition for reproductive fitness (Andersson, 1994; Andersson and Iwasa, 1996). Each individual organism is designed to maximize its reproductive fitness; a measure of the reproductive success of an individual relative to that of its conspecifics (Andersson and Iwasa, 1996). Strong competition between individuals is therefore expected when a resource, such as mates, used by one individual is consequently less available to others (Andersson, 1994; Emlen and Oring, 1977).

In species with internal fertilization, females and males differ in their strategies to maximize fitness, leading to a conflict of interests during interactions (Andersson, 1994; Andersson and Iwasa, 1996). According to Bateman's theory, females gain fitness through investing a lot of resources (time and energy) into the survival of offspring through the production of few large, energy rich gametes (eggs) and development of zygotes through maternal brood care (Andersson, 1994; Andersson and Iwasa, 1996; Rolff, 2002). In contrast, males gain fitness by increasing mating rates, investing energy into many small, energy-poor gametes (sperm) with the sole purpose of transferring genetic material to as many eggs as possible (Andersson, 1994; Andersson and Iwasa, 1996; Rolff, 2002). Because of this, females and males differ in the resources limiting their reproductive success; females by energy resources for the production of eggs, and males by the number of eggs they can fertilize, which is essentially the number of females with whom they can mate (Andersson, 1994; Andersson and Iwasa, 1996). Traditionally, populations are considered egg-limited with no sperm-limitation. Therefore, competition over mates is seen primarily among males in a population as a result of their struggle to produce a maximum number of offspring with a limited number of mates, or in other words, to monopolize mates (Dunnington, 1999; Emlen and Oring, 1977).

Several factors can influence the intensity of male-male competition for paternity of offspring in any population and the consequent mating strategies adopted by the individuals. By competing to monopolize the environmental resources that females require for reproduction, such as shelter and feeding sites, males can increase their own reproductive success. In this case, the spatial and temporal dispersion pattern of these key resources can influence the ability of males to monopolize them, and therefore resource distribution can influence the reproductive success of males and the intensity of competition among them (Emlen and Oring, 1977). This strategy of male territory defence is seen in the wide-eyed flounder, *Bothus podas* (Carvalho *et al*., 2003), in intertidal fiddler crabs, *Uca* spp., that compete for burrows in which to mate and in hermit crabs which fight for their shells (Debuse *et al*., 1999).

Population structure and dynamics can influence the level of competition in a population. Emlen and Oring (1977) emphasized the importance of the ratio of fertilizable females to sexually active males and termed this the operational sex ratio (OSR). The OSR indicates the degree of ability to monopolize of mates in a population. When there is a skewed operational sex ratio, where receptive adults of one sex significantly outnumber those of the other, intra-sexual competition in the favoured sex will intensify as mates become limiting (Zimmer, 2001). Thus the greater the imbalance in the ratio, the stronger the competition for mates among members of the majority sex (Debuse *et al*., 1999; Emlen and Oring, 1977; Zimmer, 2001). Jirotkul (2000) found male-male competition for mates in guppies, *Poecilia reticulata* more intense when the OSR was biased towards males. This suggests that either males or females could limit population growth.

The number of fertilizable females in a population may be dependent on the biology of the species. For many species, particularly in the Brachyura, the synchrony of female receptivity to mating largely influences the number of fertilizable females available in the population at any time. If all females in a population are sexually receptive concurrently, such as the intertidal amphipod *Corophium volutator* (McCurdy *et al*., 2000), breeding opportunities may be limited to only a short period of time when the females are receptive, resulting in male-male competition for females being intense only during this time and very low at other times (Mathews, 2002). However, if female receptivity to mating is asynchronous, as seen in the snow crab, *Chionoecetes opilio* (Rondeau and Sainte-Marie, 2001), males are more able to monopolize mates, increasing the overall intensity of competition (Emlen and Oring, 1977).

Competition for paternity of offspring can continue after copulation in the form of sperm competition. Sperm competition occurs when a female can store sperm, mates with more than one male and when there is a delay between copulation and fertilization (Danielsson, 1998; Parker, 1970; Parker, 1974). Andersson and Iwasa (1996) suggested that sperm competition decreased the realized reproductive rate of males compared to females, and may have been an indirect influence on the intensity of sexual selection. In species that do not store sperm, such as the spiny lobsters

Panulirus argus and *Jasus edwardsii* (MacDiarmid and Butler, 1999), the female must mate and fertilize her eggs immediately or possibly lose the sperm. With this strategy the male can easily ensure his paternity of the female's next brood. However, in species with polyandrous females, and where the female can store the sperm of several males for long periods of time, as is common in the Brachyura (Hartnoll, 1969), the male has no certainty of his contribution to egg fertilization. In these species, such as the snow crab *C. opilio* (Diesel, 1991), males may attempt to prevent or reduce the amount of sperm competition through mate guarding to prevent rival males mating with a particular female (Diesel, 1991; Smith, 1984), or seal the sperm of earlier mates off with sperm plugs as seen in the ghost spider crab, *Inachus phalangium* (Diesel, 1989).

The behavioural mating strategies adopted by individuals in a population of any species to monopolize mates and compete for limited resources to increase their reproductive fitness are therefore dependent on both environmental and biological factors (Emlen and Oring, 1977). These factors must be considered for each population of any species when theorizing about or conducting research on their reproductive strategies.

1.2 Study species

The reproductive strategies of brachyuran crabs have been the focus of many studies over the past few decades. Although most of these studies have focussed on species important to the commercial fisheries, such as *Chionoecetes* spp. (Beninger *et al*., 1993; Elner and Beninger, 1995; Moriyasu and Comeau, 1996; Paul, 1984; Sainte-Marie and Lovrich, 1994; Sainte-Marie *et al*., 2000) and the blue crab *Callinectes sapidus* (Jivoff, 2003a), studies of reproduction in commercially unimportant species have also been conducted to help further understanding of sexual selection and sperm competition in the family Hymenosomatidae (Dunnington, 1999; Hosie, 2004; Lucas, 1980; Melrose, 1975) the majid, *Inachus phalangium* (Diesel, 1986; Diesel, 1991) and the grapsid, *Metopograpsus messor* (Anilkumar *et al*., 1999).

Halicarcinus cookii Filhol (1885) (Plate 1.1) of the family Hymenosomatidae is commonly known as a pill-box or false-spider crab (McLay, 1988; Melrose, 1975). The vast majority of hymenosomatids are found in the Indo-Pacific region, most of which

inhabit Australia and New Zealand, but the family is also represented in China and Japan, Africa, and New Caledonia. One species, *Halicarcinus planatus*, is found as far as the sub-Antarctic region of the west coast of South America (Melrose, 1975). Hymenosomatids are among the smallest of the Brachyura, ranging from a carapace width of 3-26 mm (Lucas, 1980). They are characterized by their short life spans, poorly calcified carapaces which are dorsally flattened and triangular or sub-circular in shape, often forming a horizontal rostrum, and their relatively short chelipeds that are not particularly mobile (Lucas and Hodgkin, 1970a; Melrose, 1975).

Hartnoll (1969) identified two mating strategies in the Brachyura, both of which occur in hymenosomatids. Both strategies can even occur in a single species, such as *Halicarcinus ovatus* (Lucas, 1980). One strategy involves the female only being physically able to mate when soft shelled immediately after moulting, but also the need for critical timing of copulations as females are only receptive during a few days of the year. In these species growth and reproduction alternate throughout the life of the female. This strategy is found in a variety of brachyuran families including Cancridae, Grapsidae, Xanthidae and some Portunidae (Hartnoll, 1969). Mating and moulting (gorwth) are inextricably linked in soft-shell maters.

The second strategy involves separate growth and reproductive phases of the females life. In these species, females experience a terminal/pubertal moult so that they are continuously capable of producing eggs and receptive to mating in a hard shelled condition (although primiparous females may mate when soft-shelled), allowing less stringent timing of copulations as females are receptive to mating throughout the year. All growth occurs during the immature phase. This strategy occurs in the families Corystidae, Majidae and some Portunidae (Hartnoll, 1969). In a survey of Australian hymenosomatids, Lucas (1980) showed that hymenosomatid crabs are generally capable of this continuous breeding and experience a terminal/pubertal moult, during which mature features are acquired (despite evidence for copulation occurring before the pubertal moult) and the crabs no longer moult.

The genus *Halicarcinus* is found both in New Zealand and in Australia, but species tend to be endemic and with 10 of the 12 species in New Zealand being restricted to the mainland shores (Melrose, 1975). *Halicarcinus innominatus* is unique in its trans-Tasman distribution as it has recently invaded Tasmania, although this is believed to be due to human interference (Melrose, 1975). New Zealand hymenosomatids are generally sub-littoral, although *H. cookii*, *H. varius*, *H. pubescens* and *H. innominatus* can inhabit shores as high as the lower mid-littoral zone depending on algal cover (Melrose, 1975).

Halicarcinus cookii is endemic to New Zealand and is ubiquitous along the east coast of the North and South Islands, as well as the Chatham Islands and Stewart Island (McLay, 1988; Melrose, 1975). The species lives in intertidal and sub-littoral shallow water among seaweeds on the rocky shore and its microhabitat includes dense algal fronds and holdfasts (McLay, 1988; Melrose, 1975). *H. cookii* is an opportunistic carnivore and scavenger, with a diet consisting of molluscs, polychaetes (*Perinereis*, *Lumbriconereis* and *Neanthys*) and especially amphipods (McLay, 1988). Melrose (1975) found that, when deprived of prey, the crabs would also eat algae or *Zostera*.

H. cookii is typical of hymenosomatids in size. Prior to this study, males were reported to measure up to 13 mm in carapace width and females up to 8.5 mm in carapace width (McLay, 1988; Melrose, 1975). McLay (1988) stated that the high proportion of ovigerous females found at any time indicates that *H. cookii* is capable of continuous breeding and goes through a terminal/pubertal moult, after which females mate in the hard-shell condition.

1.3 Study sites

All fieldwork for the study was conducted around the Kaikoura Peninsula, but primarily First Bay (42° 25'S, 173° 42'E) on the East side of the peninsula and Atia Point (Sharks Tooth Point) (42° 25'S, 173° 41'E) on the South facing side of the peninsula (Figure 1.1, Plate 1.2). These sites were chosen after searches around the Peninsula revealed the crabs were most easily found in these areas and the areas are on different sides of the Peninsula.

Both First Bay and Atia Point are predominantly mudstone platforms with many channels and furrows. Loose rocks and stones are rare and both areas have little sediment, although some areas in First Bay had some silt build up (no more than 3 cm thick), providing a suitable substrate where *H. cookii* was easily found. These sites experience moderate to low wave exposure, First Bay being protected from the North by Seal Reef and Atia Point being protected from the west, north and east as part of Whaler's Bay. *H. cookii* was generally found low on the shore in areas that were exposed during low tides.

The crabs were found only in areas where algae was abundant and as such, numbers found in any area varied according to seasonal algae cover. First Bay is dominated primarily by the algal species *Hormosira banksii* and *Cystophora* spp. The dominant algal species at Atia Point varied between the Foliose green *Ulva* spp. after algal blooms and, intermittently, *Cystophora* spp, particularly in the more sheltered channels and furrows.

A similar community of benthic invertebrates, sympatric with *H. cookii*, were found in both sites. These included the limpet *Cellana denticulata* and gastropods, particularly *Turbo smaragdus* and in higher areas, *Melagraphia aethiops*. Numerous amphipods of the families Gammaridae and Caprellidae and isopods of the infra-orders Valvifera and Flabellifera were also present. Several echinoderm species also found in the areas include brittlestars of the family Ophiuroidea and the sea stars *Astrostole scabra*, *Patiriella* spp. and occasionally *Stichaster australis*. Other decapods found at these sites include the false-crab *Petrolisthes elongatus*, the big-handed crab *Heterozius rotundifrons*, the masking crab *Notomithrax ursus, Halicarcinus varius* and less commonly *Elamena producta*.

1.4 Study outline

The major components of the reproductive strategies of *H. cookii* are separated into three Chapters. Chapter two investigates the demography of *H. cookii* around the

Kaikoura Peninsula. This Chapter explores changes in population dynamics and sex ratios throughout the year as well as examinations of allometric growth patterns and the development of secondary sexual characteristics. Chapter three focuses on the reproductive biology of *H. cookii*, primarily focussing on females. In this Chapter, reproductive structures are examined, as well as investigations into the periodicity of brood cycles, anatomical relationships between ovary development and brood development and patterns of sperm storage. Chapter four explores the behavioural consequences of the reproductive characteristics of *H. cookii* explored in the previous two Chapters. The Chapter investigates mate recognition and choice, mate guarding and the factors that influence these. Information from the previous three Chapters will then be integrated into a general discussion (Chapter 5) of the reproductive strategies of *H. cookii*. The discussion includes comparisons with other species and implications for the sexual selection of *H. cookii*.

Plate 1.1 Dorsal view of *Halicarcinus cookii* male (above) and female (below).

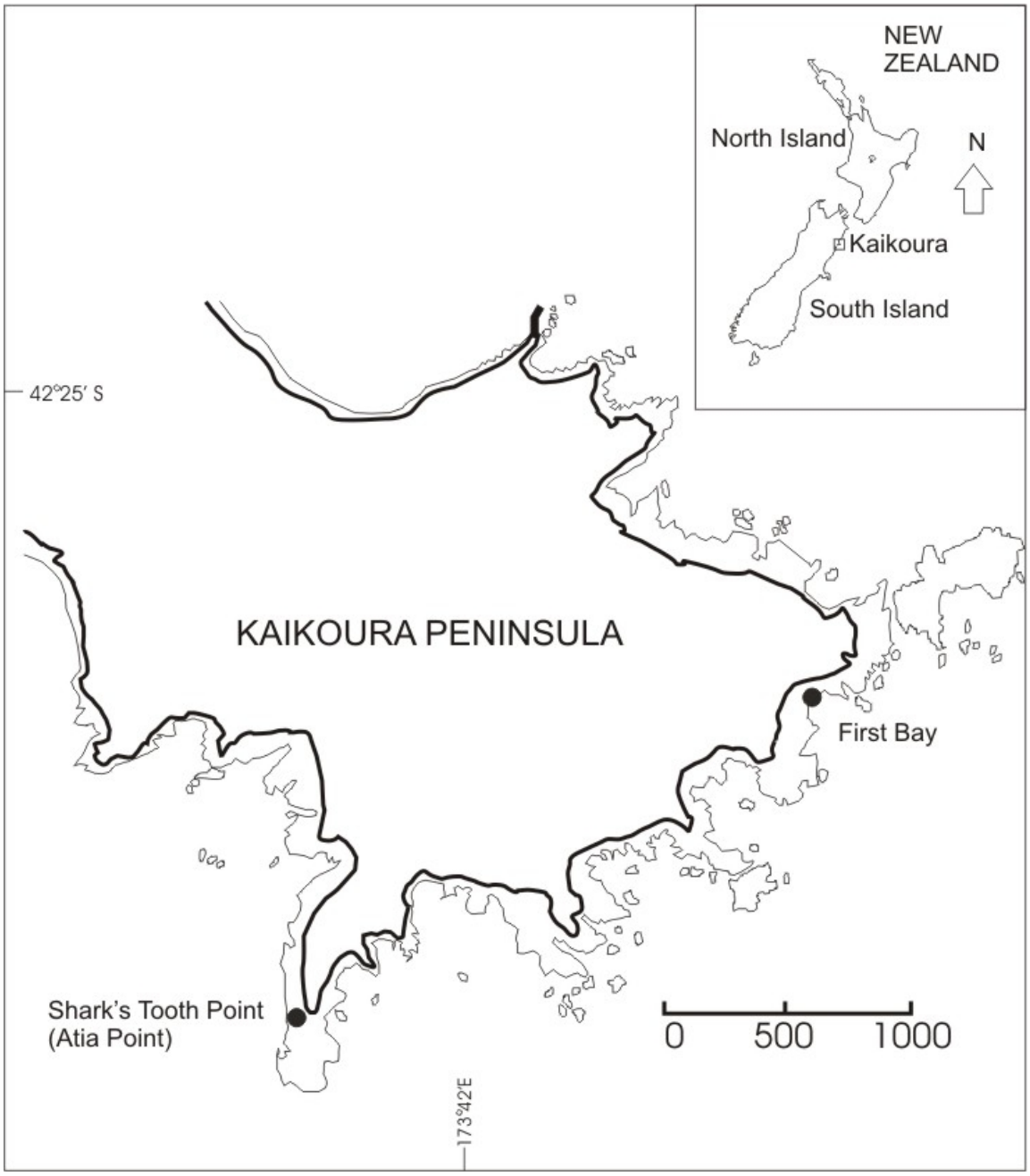
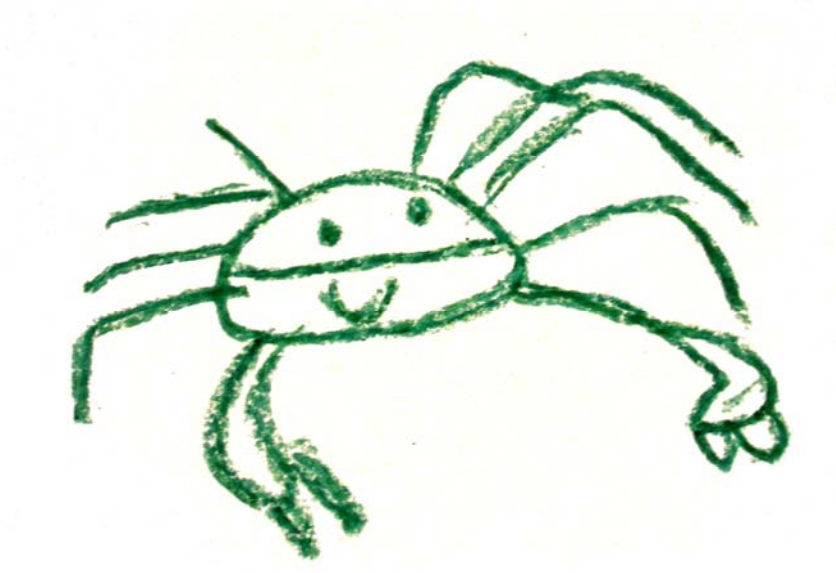


Figure 1.1 Map showing the location of primary sampling sites on the Kaikoura Peninsula. Inset shows the location of the Kaikoura Peninsula in New Zealand.

Plate 1.2 Aerial view of First Bay (above) facing East from the Kaikoura Peninsula and Sharks Tooth/ Atia Point (below) facing South from the Kaikoura Peninsula.



Chapter 2

Allometric Growth and Population Dynamics

2.1 Introduction

Studies on the relative growth of certain body dimensions that identify morphological traits which can be used to distinguish between immature and mature individuals in a population are important aspects of a species' ecology and essential when documenting its demographics and life history. The inflexible exoskeleton of crustaceans restricts growth to a succession of discrete stages at each moult (or ecdysis), where growth occurs after the old integument is shed, during each moult and before the new one hardens (Hartnoll, 1982). The nature of this discontinuous growth allows the growth and maturation of crustaceans to be easily broken down into stages, each of which have distinct relative growth rates (Hartnoll, 1982). As a crab matures, changes in its life phase (immature and mature) are indicated by changes in these growth rates (Hartnoll, 1978). When investigating morphological maturity, the most important change in life phase is when the crab becomes sexually active and is able to reproduce, this is known as the 'moult of puberty' (Hartnoll, 1969) or the 'critical moult' (Hartnoll, 1978). The pubertal moult involves the animal developing adult secondary sexual features, after which it can successfully copulate and reproduce (Hartnoll, 1969). For species with a terminal moult, such as *Halicarcinus innominatus* (Dunnington, 1999; Menzies, 1988) and *H. varius* (Hosie, 2004) the pubertal moult is the final moult, after which the crab can reproduce, but growth ceases and it no longer moults, preventing any repair to damage to its body.

In some cases, there can be a marked and disproportionate increase in the relative growth of certain secondary sexual structures compared to other structures that grow isometrically; this is known as allometric growth. Crabs commonly show a degree of allometric growth in these features compared to a reference structure (usually the carapace width) on reaching reproductive maturity. The pubertal moult is far more obvious in females than in males as it can involve allometric changes in the pleopods, sternum and most obviously, the width of the abdomen (Hartnoll, 1969). Less obviously, males of many species experience an allometric increase in chelae size at the onset of reproductive maturity (Hartnoll, 1982).

In Brachyura, the function of the abdomen differs markedly between males and females. In males the abdomen supports and covers the first two pairs of gonopods (structures used for sperm transfer during copulation). These structures change little during puberty and therefore the growth of the abdomen remains relatively isometric. In contrast, the female abdomen forms a chamber with the sternum that encloses the pleopods on which the eggs are attached. Therefore only when a female reaches maturity is there a necessity for an increased width of the abdomen, and, along with the production of eggs, a wide abdomen is a very reliable indication of female maturity (Hartnoll, 1982).

Maturity in males is more difficult to determine than in females (Hartnoll, 1965). The onset of sperm production is not a reliable indication of male maturity as this would mean that males of many species would be regarded as mature at sizes far too small to effectively mate (Comeau and Conan, 1992; Sainte-Marie and Hazel, 1992). Generally, male maturity is indicated by the size of the chelae. While male growth tends to be almost isometric even after the puberty moult, there is generally a very pronounced increase in relative size of the chelae (Hartnoll, 1982). Males often use chelae in territorial defence, combat and courtship, such as in *Uca* spp. (Hartnoll, 1969), and carrying or grasping the female during pre- or post-copulatory guarding, such as *Halicarcinus innominatus* (Dunnington, 1999; Hartnoll, 1969). This degree of positive allometry is not so pronounced in females as they do not require such weapons (Hartnoll, 1982).

Once a relatively dependable system for assessing maturity is developed, investigations into the structure and dynamics of a population can be carried out. Identifying maturity is necessary to determine the proportion of mature and immature individuals in the population, particularly the proportion of mature and reproductively active females for any given sample period. This in turn can indicate breeding seasons and operational sex ratios of the population. *Halicarcinus cookii* does not show a discrete breeding season, but Lucas (1980) suggests that reproductive effort may be more intense in one part of the year. The operational sex ratio is defined as the ratio of fertilizable females to sexually active males at any given time (Emlen and Oring, 1977), and can greatly influence the reproductive success of each sex and of the population as a whole.

There have been several population studies on hymenosomatids, including three around the Kaikoura area; two on *Halicarcinus innominatus* (Dunnington, 1999; Menzies, 1988) and one on *Halicarcinus varius* (Hosie, 2004). Others include *Elamenopsis kempi* in Iraq (Ali and Salman, 1998; Ali *et al*., 1995), *Rhynchoplax coralicola* in Japan (Gao *et al*., 1994) *Halicarcinus lacustris* (Walker, 1969) and *Halicarcinus australis* (Lucas and Hodgkin, 1970a) in Australia, and general reviews of hymenosomatids in Australia (Lucas, 1980), south-east Asia (Chuang and Ng, 1994) and New Zealand (Melrose, 1975).

In this Chapter investigations were made into the relative growth of *Halicarcinus cookii* as well as an attempt to identify the morphological traits that can be used to distinguish between males and females, and immature and mature individuals. Using this information, patterns of the structure, seasonal dynamics, recruitment, mortality and peak reproductive periods of the *H. cookii* population around the Kaikoura Peninsula were examined.

2.2 Methods:

2.2.1 Allometric Growth

To investigate allometric differences between sexually dimorphic characteristics, individual *Halicarcinus cookii*, collected from Atia Point and First Bay were brought to the laboratory and measured using Mitutoyo™ vernier callipers, accurate to 0.01 mm.

Carapace width (CW) was used as a reference dimension for comparisons of all other measurements and was measured across the widest part of the carapace (Figure 2.1, A). Abdomen width was measured across the width of the 5th segment (Figure 2.1, B and C). Although this segment is the widest abdominal segment in females, this is not the case for males, whose basal segment is the widest point. Nevertheless, the 5th segment was measured for both males and females to maintain consistency. Propodus height (PH) measured the maximum height of the largest cheliped (Figure 2.1, D). Propodus length (PL) was measured across the longest point from the carpal-propodal joint to the tip of the fixed finger of the largest cheliped (Figure 2.1, E).

Analyses of relative growth measurements were conducted using the growth equation described in Hartnoll (1982):

$$Y = aX^b,$$

where Y is the relative dimension (the dimension of interest), X is the reference dimension (CW in all cases), a is the y-intercept and b is the relative growth rate (Hartnoll 1978, 1982). The equation was altered to log-log transformed to allow for estimation of the parameters by linear regression:

$$\log Y = \log a + b(\log X).$$

Regression lines were calculated and compared using ANCOVA to identify the level and significance of allometric growth for each dimension.

19 juvenile females were collected and followed through their pubertal moult. Measurements of the carapace width (CW) and abdomen width (AW) were taken before and after the pubertal moult to produce a percentage moult increment (increase in size from immaturity to maturity). The percentage moult increment (PMI) was calculated using the formula:

$$PMI = (\text{post-moult } Y - \text{pre-moult } Y) / (\text{pre-moult } Y) \times 100$$

where Y is either CW or AW.

2.2.2 Population Dynamics

A fifteen month population survey was conducted from October 2004 to December 2005 to investigate temporal changes in population structure over at least one year. Surveys occurred over two consecutive days each month and involved a 90 minute haphazard search at both Atia Point and First Bay. Searches were focused on the mid to low shore in stony and sandy areas with abundant algal species, especially *Hormosira banksii*, *Ulva* sp. and *Cystophora retroflexa*. All *Halicarcinus cookii* individuals found were collected and brought to the laboratory. In the laboratory the crabs were measured (see above), sexed, their level of maturity determined and females were recorded as ovigerous (carrying a brood) or non ovigerous (not carrying a brood). The stage of the brood carried by ovigerous females was determined as stage 0-5 (see Chapter 3 for descriptions). Crabs were returned to their original site to avoid influencing subsequent searches.

The sex of each crab was determined by the shape of the abdomen. Male abdomens were narrow, triangular and did not form a hollow brood chamber. Adult female abdomens cover the entire sternum and form a hollow enclosure where embryos are held by the pleopods until hatching. Juvenile females were recognised by a flat abdomen not completely covering the sternum or forming a brood chamber. Although cheliped size is often considered an indication of male maturity in many brachyurans (Hartnoll, 1978; Hartnoll, 1982; Hartnoll, 1985; Lucas, 1980), there was no obvious morphological structure indicative of male maturity, so males were regarded as

immature with CW < 7 mm and as mature with CW > 7 mm based on spermatophore presence (Chapter 3) and mating observations.

Monthly samples of the population provided data for the calculation of sex ratios for each month from October 2004 until December 2005. Population sex ratios were determined by using all collected crabs for each monthly sample. Operational sex ratios were determined by only using mature females and mature males. Chapter 4 indicates that males show a preference to mate with females carrying late stage eggs (stage 5) compared to other females. In light of this, females were separated into brood stages and the operational sex ratio was recalculated to produce ratios of mature males to females carrying each brood stage.

Further examination into population sex ratios of *H. cookii* involved pooling all population data and dividing them into size classes of 2 mm increments, except for the 0-4 mm size class (due to the paucity of animals in this size class found). Sex ratios were calculated for each size class and χ^2 used to determine if they differed significantly from 1:1.

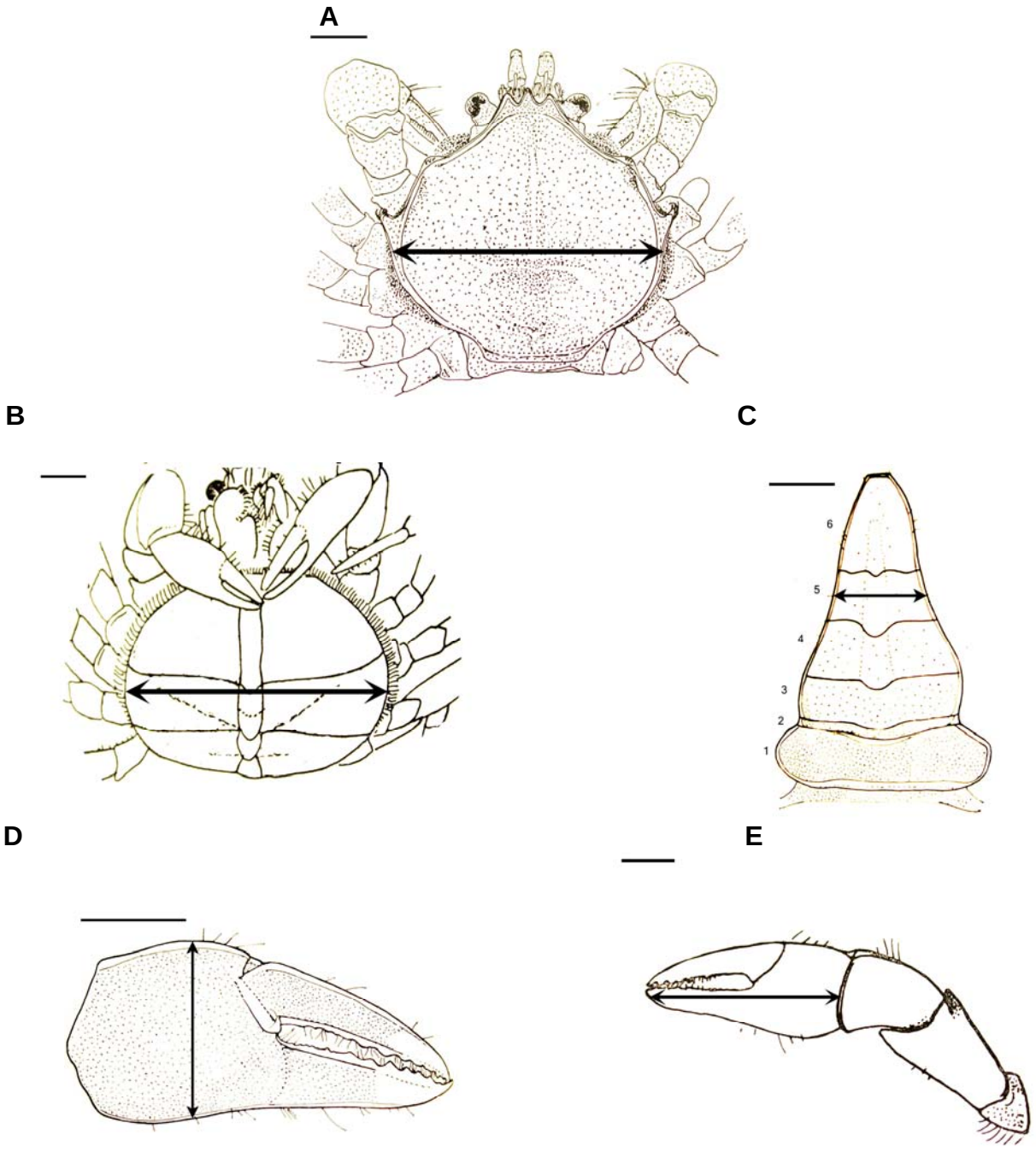


Figure 2.1 Dimensions measured to investigate relative growth of *Halicarcinus cookii*. **(A)** Carapace width (dorsal view), **(B)** Abdomen width of female, (**C**) Abdomen width of male, **(D)** Propodus height and **(E)** Propodus length. Scales represent 1 mm (from Melrose 1975).

2.3 Results

2.3.1 Allometric Growth

During the examination of allometric growth, no obvious discontinuity in growth rates in males was observed, which would suggest a single large change in increment at puberty. Therefore, all measurements for male sizes were pooled into a single group for all analyses.

Abdomen Width

Negative allometric abdomen growth was shown in mature females (b = 0.85) and males (b = 0.80). Immature females had the highest allometric growth rate in abdomen width, and were the only group to show positive allometric growth (b = 1.48) (Table 2.1).

Females showed a change in allometric abdomen growth from positive prior to the pubertal moult to negative when mature (Table 2.1, Fig. 2.2 A, B). As the maturity moult is also a terminal moult, there is no opportunity for allometric growth in the mature instar. However, mature females show an allometry of size equivalent to allometric growth and can be termed ‘apparent growth’ as a result of females undergoing the pubertal moult over a range of sizes (Hartnoll, 1982). Allometry of size in mature female abdomen width was significantly different from 1 (b = 0.85) (Table 2.1).

Regression slopes of allometric abdomen growth are significantly different from 1 in all groups (Table 2.1). Relative growth rates of AW were also significantly different between all groups (df = 1, 182 (males); 58 (immature females); 203 (mature females), $p < 0.001$ in all cases) (Table 2.2).

Propodus size

Both immature and mature females showed negative allometric growth in propodus length (b = 0.68 and 0.85 respectively) while males showed a positive allometric growth rate (b = 1.21) (Table 2.1). There appears to be little difference in propodus length between the three groups (Fig. 2.3 A, B), however, propodus length was still

significantly different between the three groups, being longest in males and shortest in immature females (df = 1, 182 (males); 58 (immature females); 203 (mature females), $p < 0.001$ in all cases) (Table 2.2).

Males showed significantly positive allometric growth in propodus height (b = 1.45) while in females, growth in propodus height changed from isometric in immature females to significantly allometric in mature females (0.89 (immature females) to 1.07 (mature females)) (Table 2.1). However, allometric growth in propodus height was significantly different between all groups (df = 1, 182 (males); 58 (immature females); 203 (mature females), $p < 0.001$ in all cases) (Table 2.2).

There was no distinct grouping in the relationship between propodus height and carapace width in males (Fig. 2.4), as is common in brachyurans, so there was no indication of possible male maturity. An arbitrary criterion was therefore established to separate mature from immature males for convenience. In Chapter 3, results of male dissections suggested that males in a size range of 6-8 mm CW began to produce spermatophores. Therefore in this study, where data for males are not pooled, male *H. cookii* will be regarded as immature with a CW < 7 mm and mature with CW > 7 mm. However, Dunnington (1999) showed that male maturity could not be defined by CW alone in *H. innominatus*. The morphology of the chelae, particularly the development of a tooth on the dactyl of the chelae, was also used to assign maturity to males. Male *H. cookii* do not develop such a tooth (Melrose, 1975). Furthermore, there was a substantial overlap in female CW over the maturity moult. Immature females ranged in CW from 4.05-9.01 mm while mature females ranged from 5.44-11.51 mm. This suggests that males may also mature over a range of CW so that the estimates of male maturity established above are likely to be a rough guide rather than a reliable criterion.

Table 2.1 Equations for linear regressions of relative growth of abdomen width (AW), propodus length (PL) and propodus height (PH) for *Halicarcinus cookii*. a = y-intercept, b = allometric growth rate, R^2 = determination coefficient, n = sample size, F = F-value, S = significant ($p < 0.05$) and NS = not significant ($p > 0.05$).

Dimension	Regression Equation $Log_{10}Y=(b)log_{10}X+log_{10}a$	R^2	n	F-value	Significance of Allometry
Abdomen Width					
Females	$log_{10}AW = (0.8495)log_{10}CW + 0.1508$	0.84	205	36.28	S -
Immature females	$log_{10}AW = (1.4755)log_{10}CW - 0.6245$	0.79	62	59.49	S +
Males	$log_{10}AW = (0.7993)log_{10}CW - 0.5334$	0.84	184	550.6	S -
Chela					
Propodus Length					
Females	$log_{10}PL = (0.8483)log_{10}CW - 0.1295$	0.64	205	9.9	S -
Immature females	$log_{10}PL = (0.6784)log_{10}CW + 0.0126$	0.66	62	0.06	NS
Males	$log_{10}PL = (1.2113)log_{10}CW - 0.362$	0.94	184	516.81	S +
Propodus Height					
Females	$log_{10}PH = (1.0747)log_{10}CW - 0.8009$	0.66	205	252.12	S +
Immature females	$log_{10}PH = (0.892)log_{10}CW - 0.6168$	0.67	62	87.06	S -
Males	$log_{10}PH = (1.4518)log_{10}CW - 0.9462$	0.93	184	1414.66	S +

Table 2.2 Analysis of covariance of allometric growth rates of abdomen width, propodus length and propodus height of both male and female *H. cookii*. F = F statistic, df = degrees of freedom, p = level of significance.

Dimension	F-value	df	p-value
Abdomen Width	111.61	2, 447	$p < 0.001$
Propodus Length	320.473	2, 447	$p < 0.001$
Propodus Height	1929.335	2, 447	$p < 0.001$

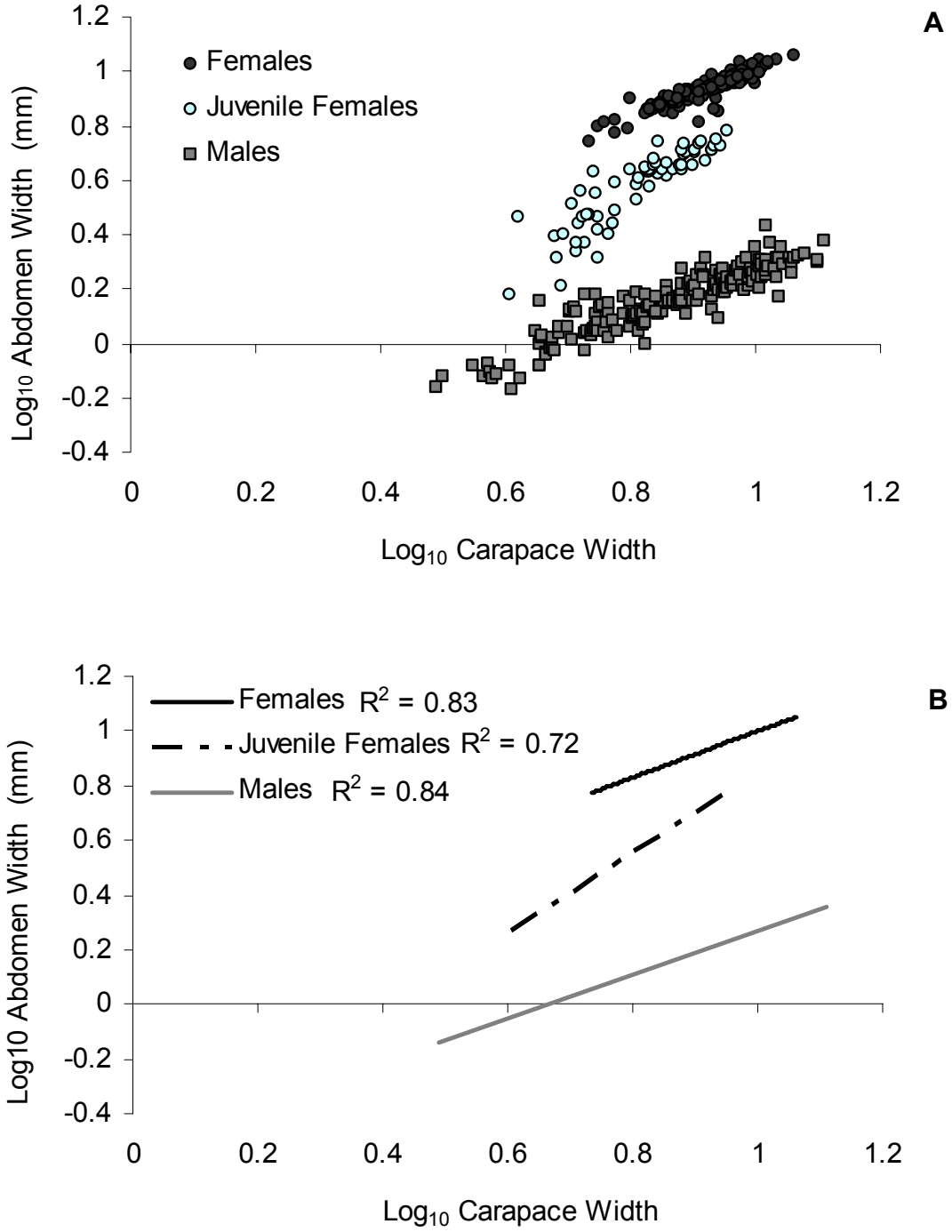


Figure 2.2 Allometric growth rate of the abdomen width to carapace width in *H. cookii*. **(A)** Log_{10} abdomen width plotted against log_{10} carapace width, n = 184 males, 205 mature females and 62 immature females. **(B)** Regression lines of data shown in (A), R^2 values are given, regression equations shown in Tables 2.1.

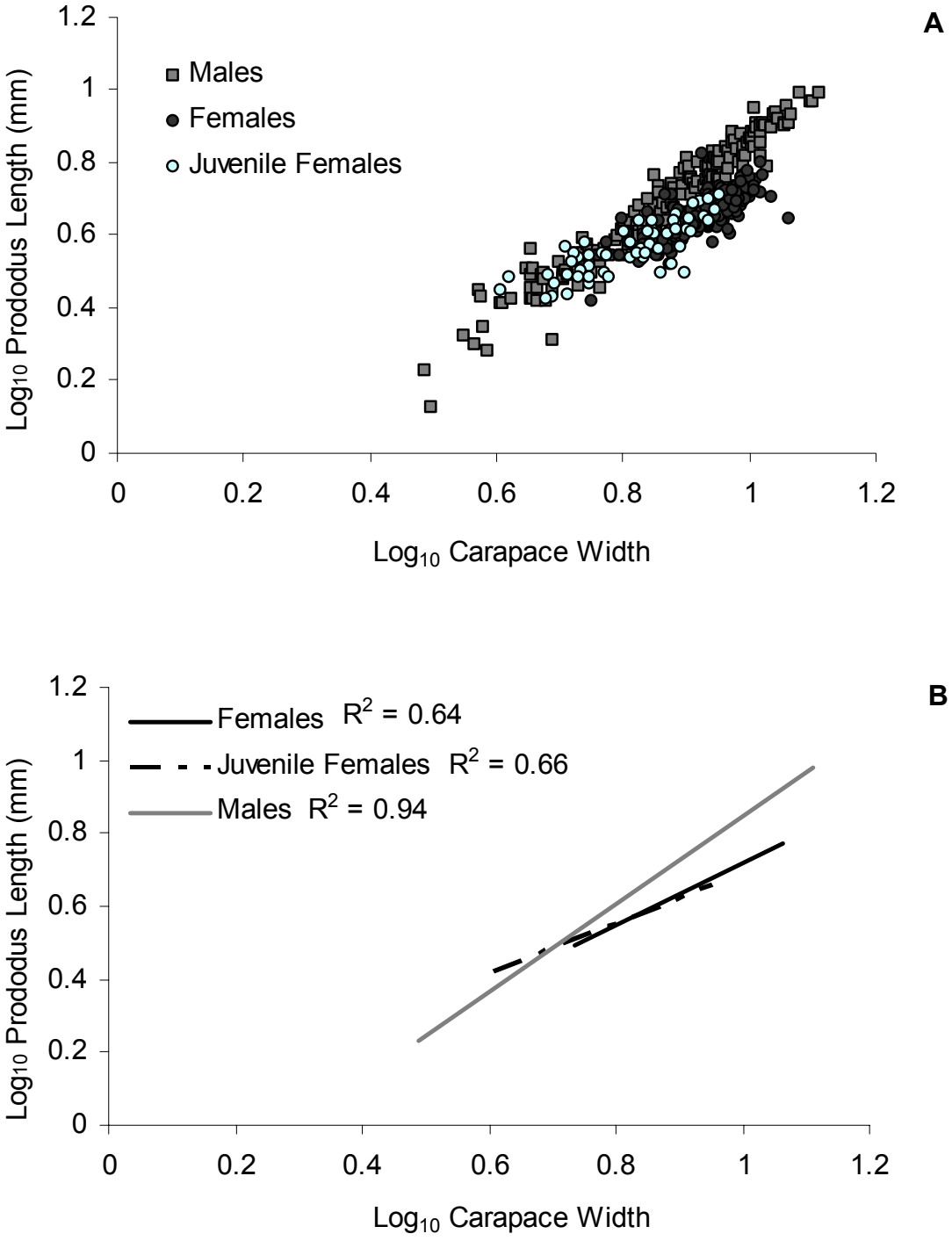


Figure 2.3 Allometric growth rate of propodus length to carapace width in *H. cookii*. **(A)** Log_{10} propodus length plotted against log_{10} carapace width, n = 184 males, 205 mature females and 62 immature females. **(B)** Regression lines of data shown in (A), R^2 values are given, regression equations shown in Tables 2.1.

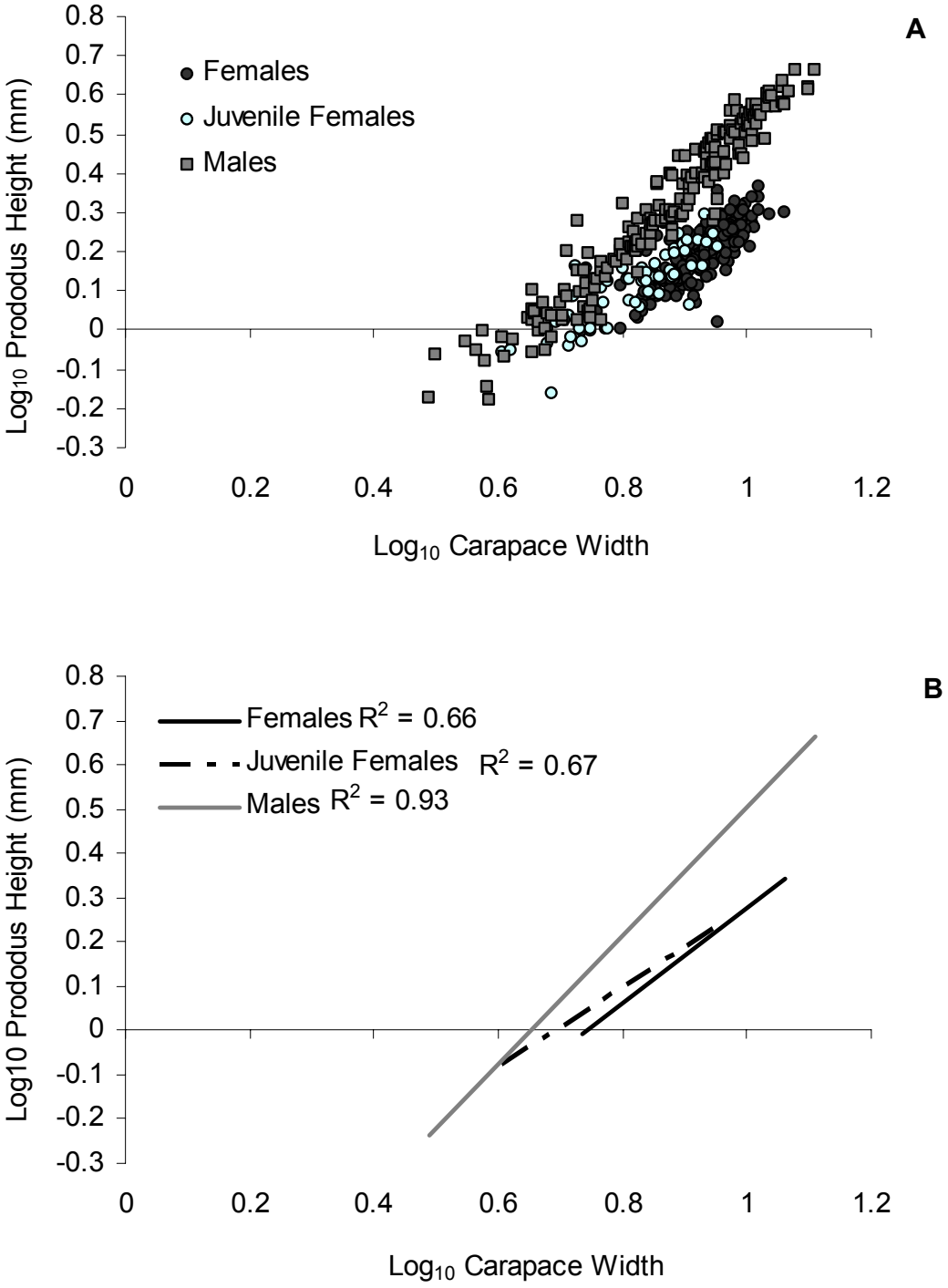


Figure 2.4 Allometric growth rate of propodus width to carapace width in *H. cookii*. **(A)** Log_{10} propodus width plotted against log_{10} carapace width, n = 184 males, 205 mature females and 62 immature females. **(B)** Regression lines of data shown in (A), R^2 values are given, regression equations shown in Tables 2.1.

Female Pubertal Moult

By monitoring 19 females in their penultimate instar through their maturity moult, it was possible to produce the percentage moult increments (PMI) for the carapace width and abdomen width. The PMI for carapace width ranged from 5.8%-41.29% with a mean of 17.96% (±2.55). This suggests that females increased close to 20% in overall size over the pubertal moult. In contrast, the PMI for abdomen width ranged from 46.115-146.58% with a mean of 96.13% (±6.02). Therefore, while increasing in overall size by approximately 20%, female increased in abdomen width by close to 100%.

A log transformation allowed a comparison between these measurements and the data collected for allometric growth. A plot of measurements of pre and post-pubertal moult females with the regression lines from the allometric growth study (Fig 2.2 B) shows that females monitored through their pubertal moult in the laboratory fit the measurements of allometric growth of females found in the field (Fig 2.5).

Assuming the relationship between PMI and CW is linear, it is possible now to more accurately determine the range over which immature females moult to maturity. By selecting the smallest recorded mature female CW (5.44 mm), and using the formula:

$$\text{Immature CW} = (\text{Mature CW})/(1+(\text{PMI}/100))$$

the CW of the smallest penultimate can be calculated to be 4.61 mm, rather than the 5.51 mm CW of the smallest penultimate female found in the field. This calculation provides a range of penultimate instar CW of 4.61-9.01 mm.

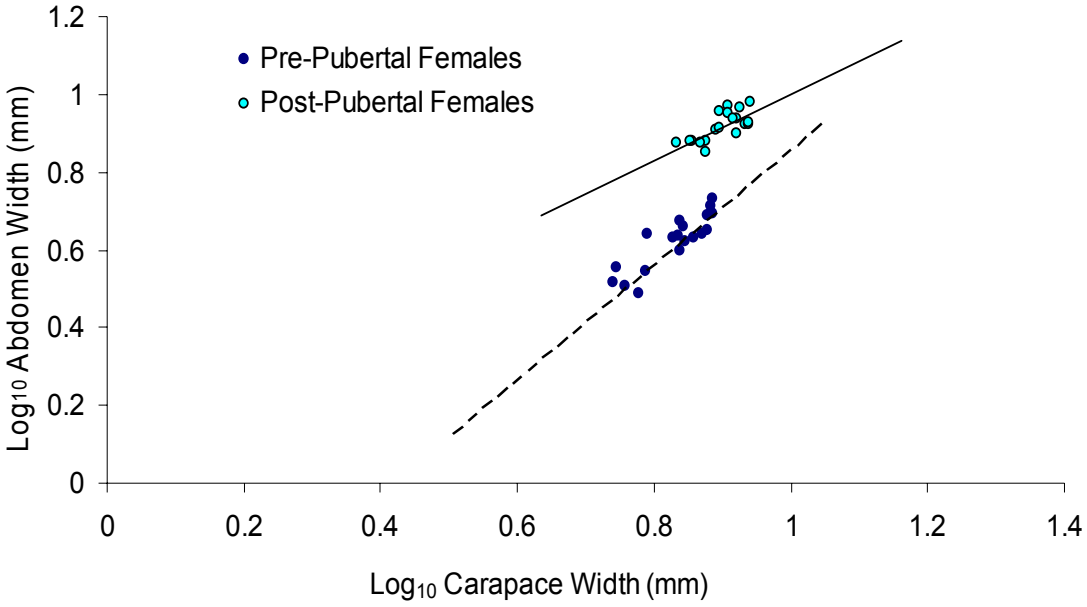


Figure 2.5 Comparisons of the $\log_{10}$ carapace width to $\log_{10}$ abdomen width of females before and after their pubertal moult. N = 19. Regression lines are taken from allometric growth rates (Fig. 2.2). Regression equations and R^2 values are shown in Table 2.1.

2.3.2 Population Dynamics

Size Frequencies

Throughout the year, the mean CW showed a similar trend in both males and females. There was a slight decrease in mean CW during the winter months from a peak of 9.50 mm in males in November 2004, and 9.25 mm in females in December 2004 to as low as 6.74 mm in females in March 2005 and 6.57 mm in males in July 2005 (Fig. 2.6).

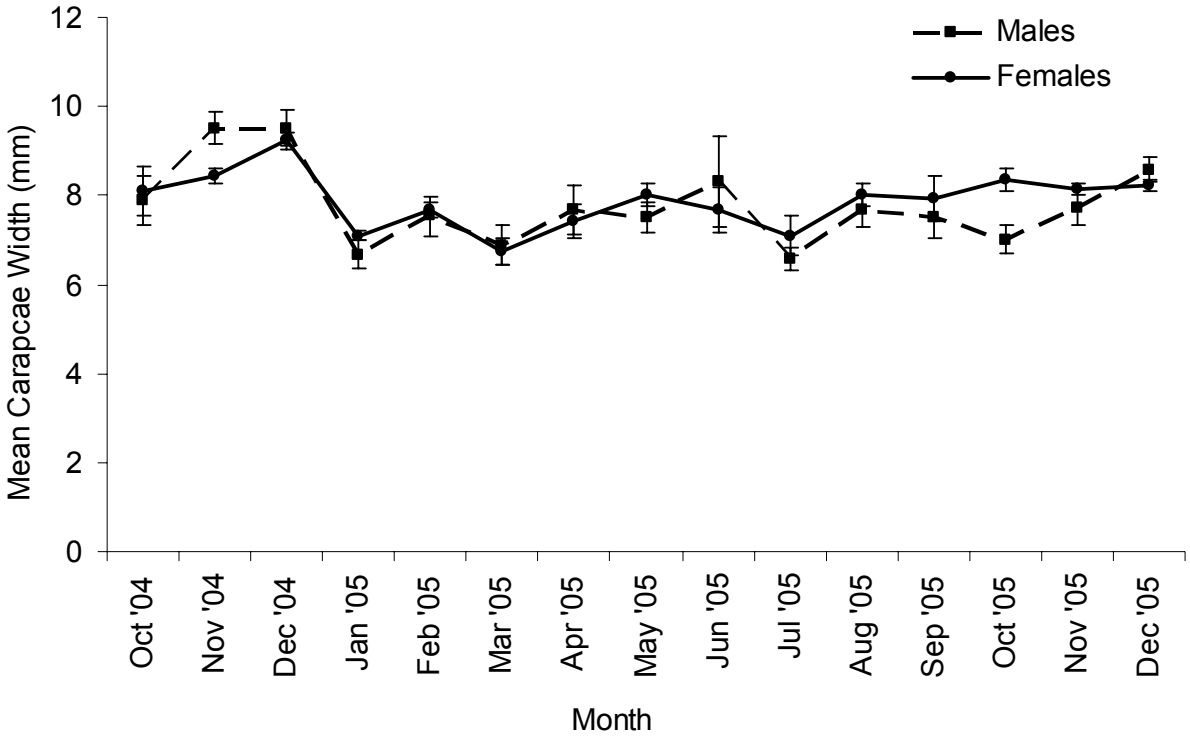


Figure 2.6 Mean carapace width (±1 S.E) of male and female *H. cookii* sampled monthly from October 2004 to December 2005.

There was a range of size classes found throughout the year. Although a higher percentage of individuals in the smaller size classes were found in mid-winter (June-July 2005), small individuals were found throughout the year (Fig. 2.7).

There was a general trend for a unimodal size distribution in both males and females throughout the year. Carapace width in both females and males was generally unimodal at 6.1-8 mm. Males showed unimodal carapace width being in October 2004 (4.1-8 mm), December 2004 (10.1-12), October 2005 (4.1-8 mm) November 2005 (4.1-6 mm) and December 2005 (8.1-10 mm) (Fig. 2.7).

Some monthly samples showed two size groups, particularly in the winter months, suggesting a period of increased recruitment. In females, this size distribution occurred in July 2005 (at 4.1-6 mm (all immature) and 8.1-10 mm (all mature)), while in males

this occurred in April and May 2005 (at 4.1-6 mm and 8.1-10 mm), June 2005 (4.1 mm and 10.1-12 mm) and July 2005 (2.1-4 mm and 10.1-12 mm) (Fig. 2.7).

Combining all the size classes throughout the year, it was possible to examine the sex ratio according to size. Males peaked in the percentage of the population in two separate size classes with the highest percentage of individuals with carapace width 2.1-6 mm and 10.1-14 mm and occupied 100% of the 12.1-14 mm size class. The largest male found was 12.92 mm CW. Females dominated the middle size classes from 6.1-10 mm CW with the largest individual recorded as 11.51 mm CW and a mean of 8.4 mm CW. Only one individual was found in the 0-2 mm size class (CW = 1.99 mm), at this size sex is often difficult to determine, so no valid conclusions can be made regarding this size class (Fig. 2.8). Sex ratios were significantly different from 1:1 in the 6.1-8 mm and 8.1-10 mm size classes where females were the majority ($p < 0.001$ in both cases), and in the 10.1-12 mm size class where males were the majority ($p < 0.05$) (Table 2.3).

Maturity

Immature females covered a range of size classes throughout the year. The smallest size class in which immature females were found was 2.1-4 mm CW in March and April 2005. The largest size class occupied by immature females (8.1-10 mm CW) occurred in June, August and November 2005 (Fig. 2.7). A similar observation could not be made for males due to the inability to accurately distinguish between immature and mature males (described above).

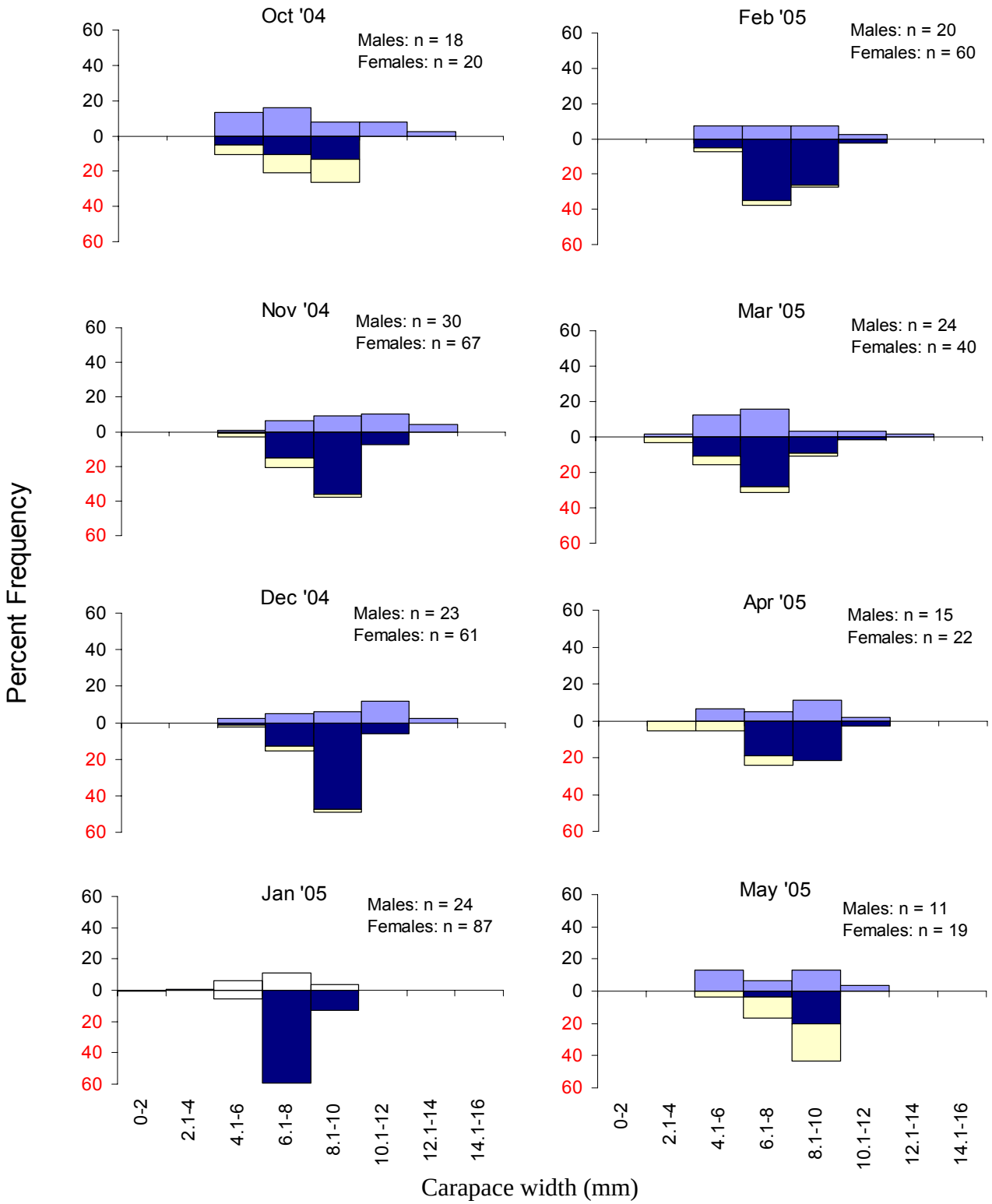


Figure 2.7 Histogram of size frequency of the *Halicarcinus cookii* population sampled from October 2004 to December 2005.

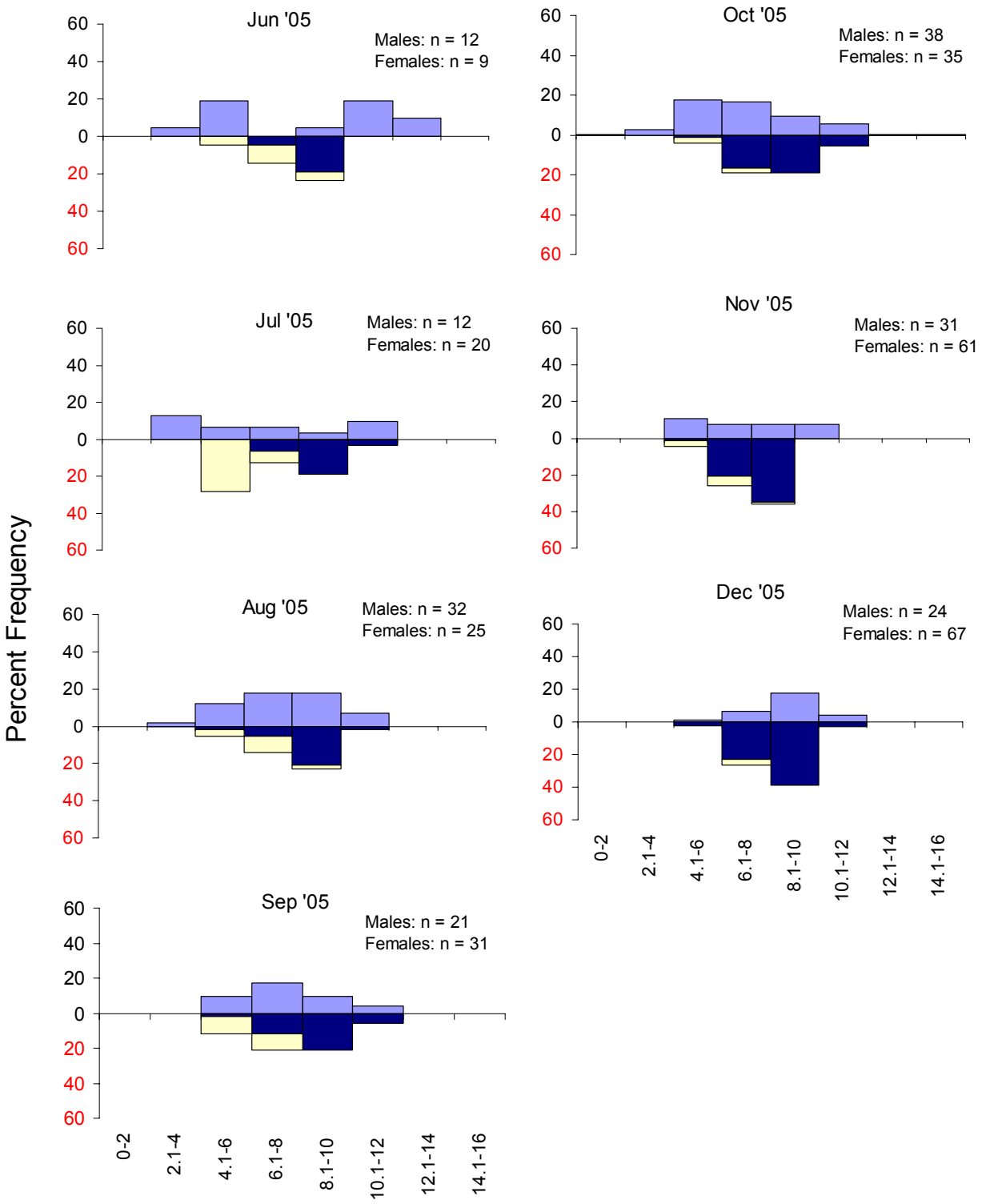


Figure 2.7 Continued. Size frequency of the *Halicarcinus cookii* population sampled from October 2004 to December 2005.

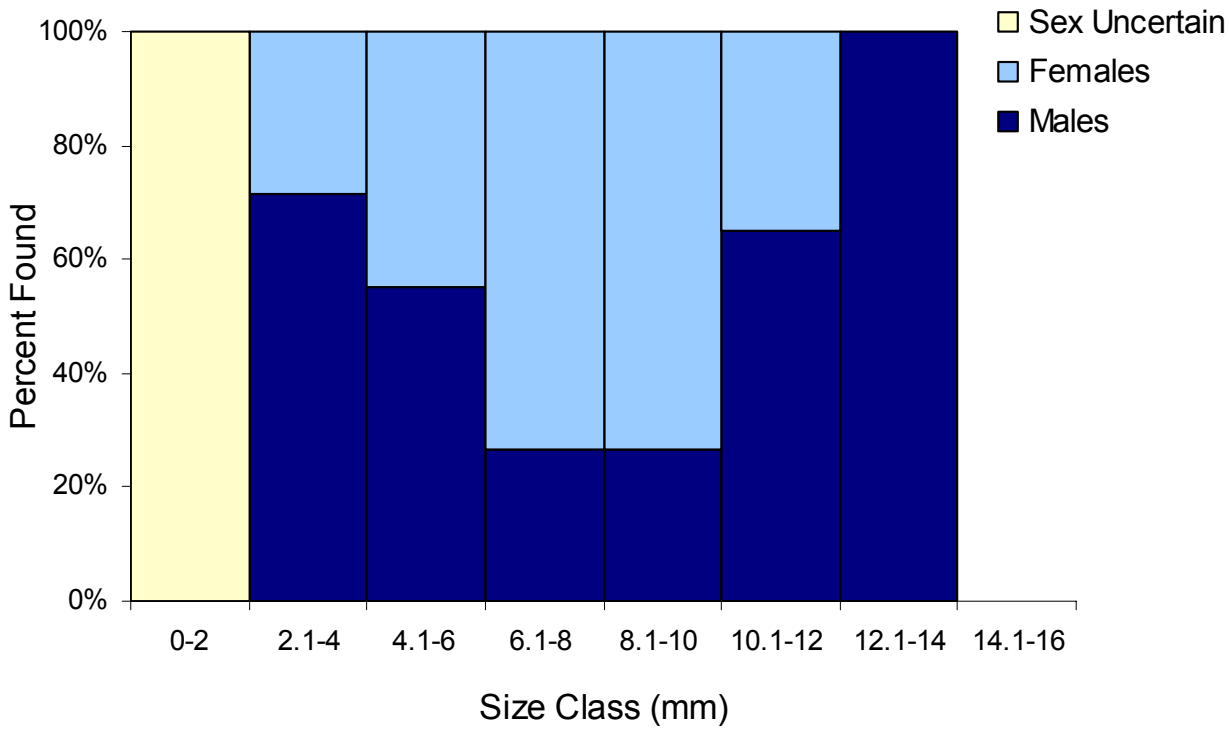


Figure 2.8 Percentage of the population of *H. cookii* occupying each size class according to sex.

Table 2.3 Sex ratios as a function of size for the population of *H. cookii* sampled over fifteen months (October 2004-December 2005). Ratios are males per female. The significance of ratios differing from 1:1 are indicated as NS = not significant, * = $p < 0.05$. *** = $p < 0.001$.

Size	Males	Females	Total	Sex Ratio	Significance
2.1-4 mm	10	5	14	2:1	NS
4.1-6 mm	63	51	114	1.2:1	NS
6.1-8 mm	76	208	284	0.4:1	***
8.1-10 mm	69	191	260	0.4:1	***
10.1-12 mm	37	20	62	1.9:1	*
12.1-14 mm	5	0	5	All Males	

For analysis of population structure and sex ratios, males were separated into immature and mature individuals according to the arbitrary method described above, with males with CW > 7 mm regarded as immature, and those with CW > 7mm as mature to compensate for the lack of reliable criteria to distinguish male maturity. During the summer months, the population was dominated by mature individuals in both sexes. The highest percentage of the population occupied by mature males (33.3%) occurred in August 2005, and the lowest (14.4%) occurred in January 2005. Immature males were at their highest percentage of the population in October 2004 (27%) and through the winter months of 2005 (22.7% in June through to 24.7% in October) (Table 2.4, Fig. 2.9). Mature females were most common in spring and summer, reaching a peak of 77.5% of the population in January 2005 and were least common in the winter months at a low of 22.7% in June 2005. Immature females showed the opposite trend to mature females, being most abundant in May 2005 (41.4%) and at a low in January 2005 (0.9%) (Table 2.4, Fig. 2.9).

Table 2.4 Population composition of *H. cookii* sampled monthly from October 2004 to December 2005.

Month	Mature Males	Immature Males	Mature Females	Immature Females
Oct '04	21.6	27.0	24.3	27.0
Nov '04	27.3	5.1	57.6	10.1
Dec '04	21.6	2.0	72.5	3.9
Jan '05	14.4	7.2	77.5	0.9
Feb '05	17.5	7.5	68.8	6.3
Mar '05	23.4	14.1	50.0	12.5
Apr '05	27.0	13.5	43.2	16.2
May '05	20.7	13.8	24.1	41.4
Jun '05	31.8	22.7	22.7	22.7
Jul '05	15.6	21.9	28.1	34.4
Aug '05	33.3	22.8	28.1	15.8
Sep '05	18.9	20.8	41.5	18.9
Oct '05	27.4	24.7	42.5	5.5
Nov '05	20.9	13.2	56.0	9.9
Dec '05	25.3	4.2	67.4	3.2

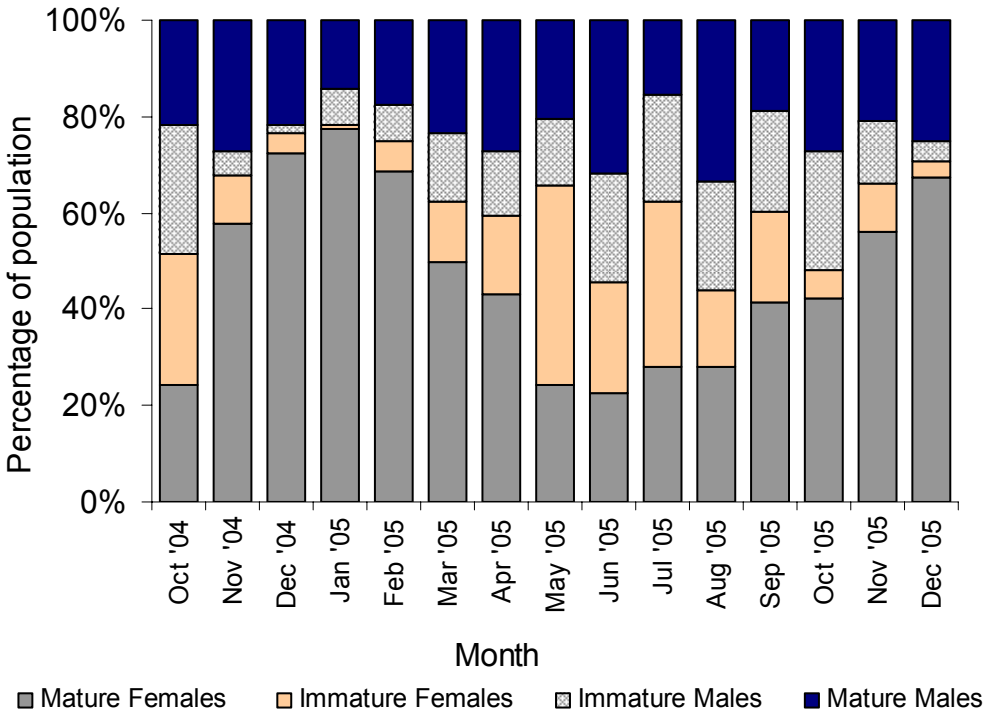


Figure 2.9 Percentage of the population of *H. cookii* sampled from October 2004 to December 2005 according to reproductive status (mature/immature males/females).

Ovigerous Females

Mature females were divided according to whether or not they carried eggs (ovigerous or non-ovigerous), and the stage of development of the brood they were carrying when found in the field (see Chapter 3 for descriptions of brood stages). Of the mature females found, a mean of 96.4% (±1.07%) were ovigerous throughout the year. The percentage of females that were ovigerous remained high and relatively constant between months, ranging from 85.9% in December 2005 to 100% in October 2004, March-July 2005 and September 2005 (Table 2.5, Fig. 2.10). Most ovigerous females were carrying stage 1 broods (44.4% ±3.17), followed by those carrying stage 2 broods (16.4% ±2.13%), stage 5 broods (14.1% ±1.33), stage 3 broods (11.6% ±2.04), and those carrying stage 4 broods were the least common (9.8% ±1.38). Therefore, assuming the sampling was unbiased, it can be estimated that, of the total brood development time, stage 1 lasts for 44%, followed by stages 2 at 16% and 5 as 14%, and stages 3 and 4 would have the shortest durations of 12% and 10% respectively.

Table 2.5 Composition of the mature female population of *H. cookii* sampled according to brood stage. Values are presented as percentages of mature females found.

Month	Stage 0	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
Oct '04	0.0	66.7	22.2	0.0	0.0	11.1
Nov '04	7.0	56.1	8.8	7.0	7.0	14.0
Dec '04	5.4	29.7	16.2	16.2	12.2	20.3
Jan '05	5.8	50.0	16.3	5.8	10.5	11.6
Feb '05	3.7	48.1	13.0	9.3	11.1	14.8
Mar '05	0.0	34.4	21.9	3.1	21.9	18.8
Apr '05	0.0	43.8	6.3	18.8	12.5	18.8
May '05	0.0	28.6	14.3	28.6	14.3	14.3
Jun '05	0.0	20.0	40.0	20.0	0.0	20.0
Jul '05	0.0	44.4	22.2	22.2	11.1	0.0
Aug '05	6.3	56.3	6.3	6.3	6.3	18.8
Sep '05	0.0	54.5	13.6	9.1	9.1	13.6
Oct '05	6.5	48.4	16.1	6.5	9.7	12.9
Nov '05	5.9	45.1	13.7	11.8	11.8	11.8
Dec '05	14.1	40.6	15.6	9.4	9.4	10.9

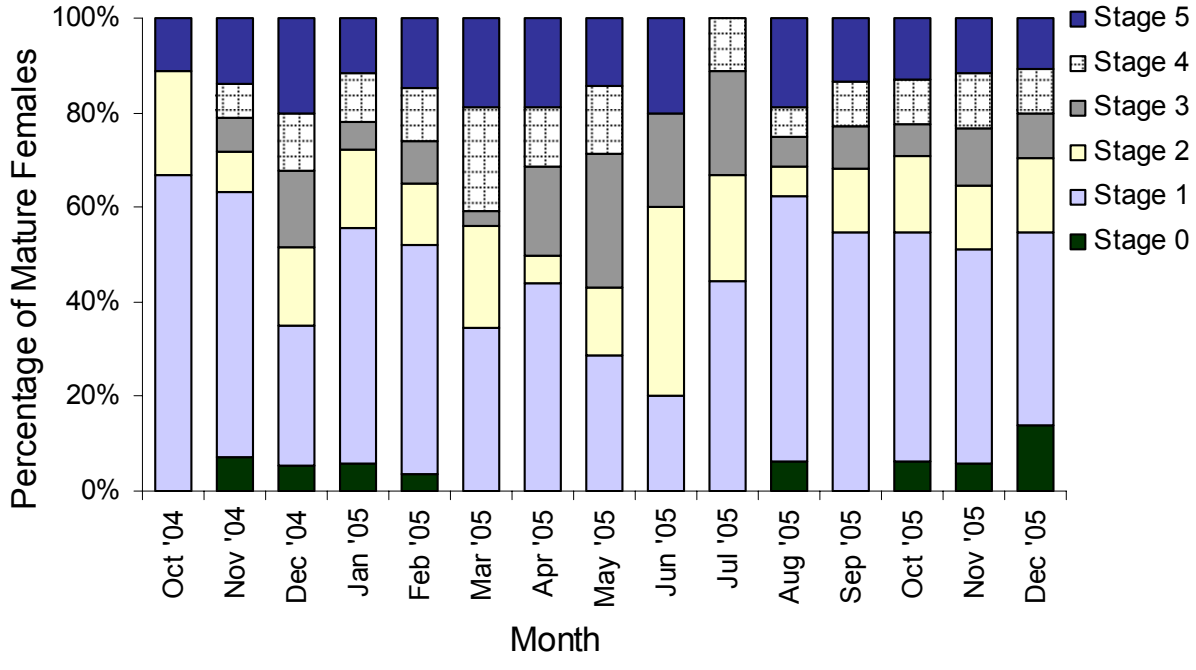


Figure 2.10 Composition of the mature female population of *H. cookii* sampled from October 2004 to December 2005 according to brood stage (0-5).

Sex Ratios

During the warmer months between November 2004 and May 2005 and November to December 2005, all sex ratios differed significantly from 1:1 ($p < 0.001$ in all months but March 2005 when $p < 0.05$) except in April and May 2005 ($p > 0.05$ in both cases) (Table 2.6). During this period population sex ratios were consistently below 1 male per female. The sex ratio was at its lowest during the height of summer, lowering to 0.28 males per female in January 2005 and increasing to 0.68 males per female in April 2005 (Fig. 2.11).

There was considerable fluctuation in population sex ratios during the colder months of October 2004 and June-October 2005 (Fig. 2.11). Ratios ranged from 0.6 males per female in July 2005 to 1.28 males per female in August 2005, but no population sex ratios during this time were significantly different from 1:1 ($p > 0.05$ in all cases) (Table 2.6).

Operational sex ratios differed significantly from 1:1 in December 2004 to April 2005 ($p < 0.001$ in January to March 2005 and $p < 0.05$ in December 2004 and April 2005) and November and December 2005 ($p < 0.001$ in both cases) (Table 2.7). The operational sex ratio (mature males per mature female) showed a similar trend to the population sex ratio, remaining below 1:1 during the warmer months between November 2004 and May 2005, decreasing to a low of 0.09 mature males per mature female in February 2005 and peaking at 0.86 males per female in May 2005 (Fig. 2.11).

Like the population sex ratio, the operational sex ratio became more erratic during the colder months of October 2004 and June-October 2005) ranging from 1.4 mature males per mature female in June 2005 to 0.45 males per mature female in September 2005 (Fig. 2.11). No operational sex ratios were significantly different from 1:1 during this cooler period, except in September 2005 ($p < 0.05$) (Table 2.7).

In Chapter 4, the difference in attractiveness of females carrying different brood stages is compared and discussed. Consequently, an investigation into the degree of limitation

of females carrying each brood stage throughout the year was conducted. More stage 1 females were found than males in November 2004, January to February 2005, September 2005 and November to December 2005. Females carrying all other brood stages were outnumbered by males throughout the year (Fig 2.12).

Sample sizes were too small to validate a χ^2 test in October 2004 and between March and October 2005, so these months were excluded from the calculations. Throughout the year, ratios were always significantly different from 1:1 for stage 1 females. The ratio of males per stage 1 female was only significant in January 2005 at 0.37 males per female. The same ratio with stage 2 females was always significant except in December 2004 to February 2005. The ratio of males per stage 3 females was significantly different from 1:1 in all months except December 2004. The ratio of males to stage 4 females was significant except in January and February 2005. Similarly, the ratio of males per stage 5 females was significant in all months excluding those from December 2004 to February 2005 (Table 2.8).

Table 2.6 Comparisons of population sex ratios sampled monthly from October 2004 to December 2005. Ratios differing from 1:1 are indicated as: NS = not significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. χ^2 = chi-square value.

Month	Population Sex Ratio (Males per Female)	Males	Females	Total	χ^2	Significance
Oct '04	0.95	18	19	37	0.03	NS
Nov '04	0.48	32	67	99	12.37	***
Dec '04	0.31	24	78	102	28.59	***
Jan '05	0.28	24	87	111	37.76	***
Feb '05	0.33	20	60	80	20	***
Mar '05	0.60	24	40	64	4	*
Apr '05	0.68	15	22	37	1.32	NS
May '05	0.53	10	19	29	2.79	NS
Jun '05	1.20	12	10	22	0.18	NS
Jul '05	0.60	12	20	32	2	NS
Aug '05	1.28	32	25	57	0.86	NS
Sep '05	0.66	21	32	53	2.28	NS
Oct '05	1.09	38	35	73	0.12	NS
Nov '05	0.52	31	60	91	9.24	**
Dec '05	0.42	28	67	95	16.01	***

Table 2.7 Comparisons of operational sex ratios sampled monthly from October 2004 to December 2005. Ratios differing from 1:1 are indicated as: NS = not significant, * = $p < 0.05$, *** = $p < 0.001$. χ^2 = chi-square value.

Month	Operational Sex Ratio (Males per Female)	Males	Females	Total	χ^2	Significance
Oct '04	0.89	8	9	17	1.86	NS
Nov '04	0.47	27	57	84	0.06	NS
Dec '04	0.31	22	74	96	5.36	*
Jan '05	0.28	16	86	102	28.17	***
Feb '05	0.09	14	55	69	48.03	***
Mar '05	0.47	15	32	47	24.36	***
Apr '05	0.63	10	16	26	6.15	*
May '05	0.86	6	7	13	1.38	NS
Jun '05	1.40	7	5	12	0.08	NS
Jul '05	0.56	5	9	14	0.33	NS
Aug '05	0.69	19	16	35	1.14	NS
Sep '05	0.45	10	22	32	4.5	*
Oct '05	0.65	20	31	51	2.37	NS
Nov '05	0.37	19	51	70	14.63	***
Dec '05	0.38	24	64	88	18.18	***

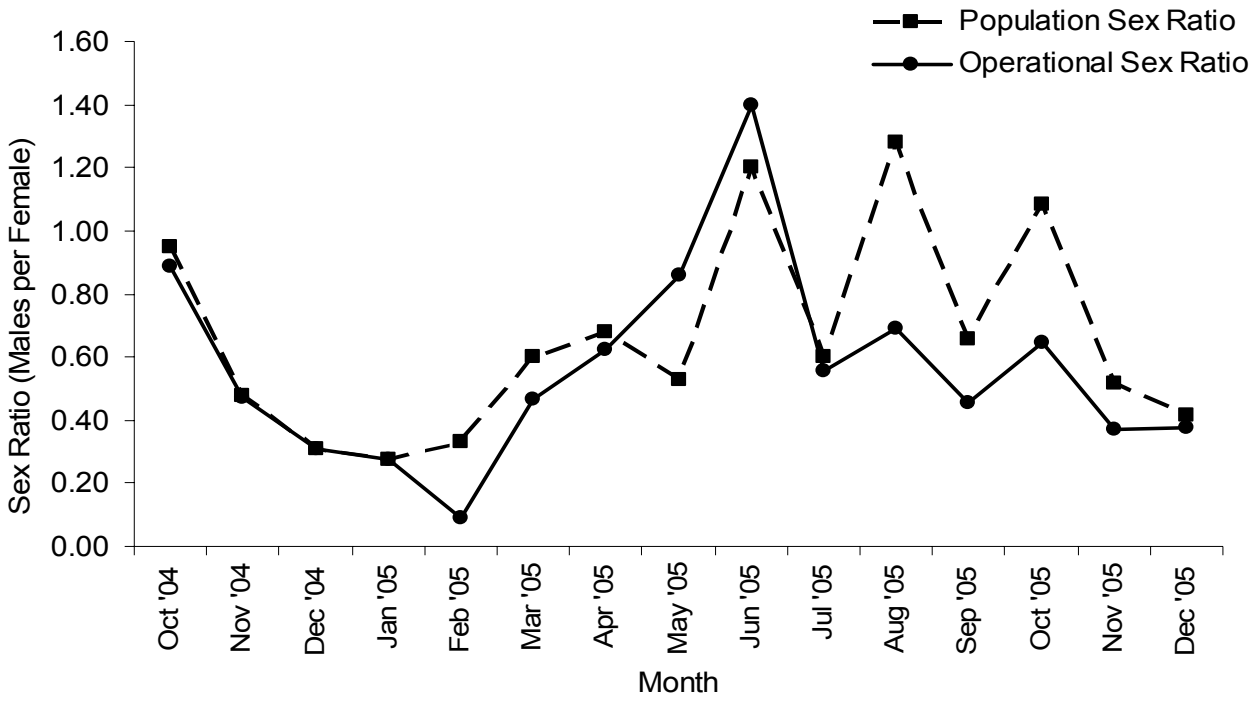


Figure 2.11 Comparison of population sex ratios and operational sex ratios of *H. cookii* each month from October 2004 to December 2005. Ratios are presented as males per female.

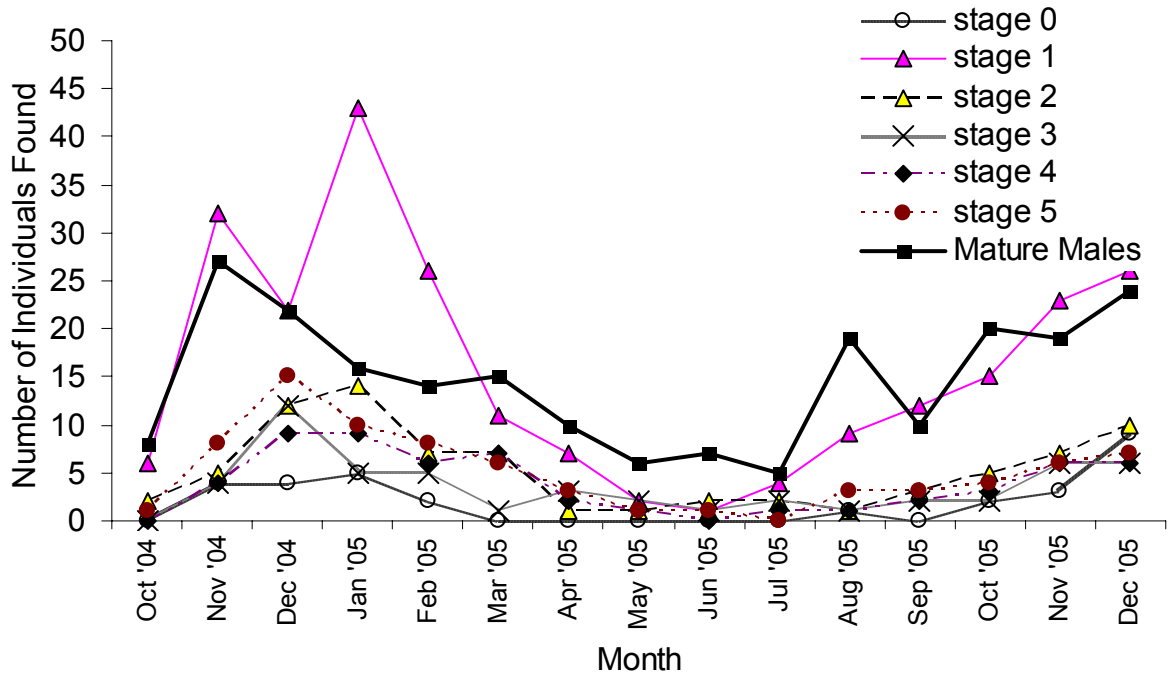


Figure 2.12 Numbers of *H. cookii* males and females according to brood stage (0-5) found each month from October 2004 to December 2005 according to brood stage carried.

Table 2.8 Comparisons of the monthly operational sex ratios according to the number of females carrying each brood stage found from November 2004 to December 2005. Sample sizes from March to August 2005 were too small to validate a χ^2 analysis and were therefore excluded. Sex ratios are presented as males per female. Ratios differing from 1:1 are indicated as: NS = not significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

Brood Stage		Month						
		Nov '04	**Dec '04**	**Jan '05**	**Feb '05**	**Oct '05**	**Nov '05**	**Dec '05**
Stage 0	Ratio	6.75	5.25	3.20	7.00	10.00	6.33	2.67
	χ^2 Value	17.06***	11.56***	5.76*	9**	14.72***	11.64***	6.82**
Stage 1	Ratio	0.84	0.95	0.37	0.54	1.33	0.83	0.92
	χ^2 Value	0.42 NS	0.02 NS	12.36***	3.6 NS	0.71 NS	0.38 NS	0.08 NS
Stage 2	Ratio	5.40	1.75	1.14	2.00	4.00	2.71	2.40
	χ^2 Value	15.13***	2.45 NS	0.13 NS	2.33 NS	9**	5.54*	5.76*
Stage 3	Ratio	6.75	1.75	3.20	2.80	10.00	3.17	4.00
	χ^2 Value	17.06***	2.45 NS	5.76*	4.26*	14.73***	6.76**	10.8**
Stage 4	Ratio	6.75	2.33	1.78	2.33	6.67	3.17	4.00
	χ^2 Value	17.06***	4.8*	1.96 NS	3.2 NS	12.57***	6.76**	10.8**
Stage 5	Ratio	3.38	1.40	1.60	1.75	5.00	3.17	3.43
	χ^2 Value	10.31**	1 NS	1.38 NS	1.64 NS	10.67**	6.76**	9.32**

2.4 Discussion

The ability to produce gametes is often used to define sexual maturity in brachyurans. Gamete production in females is easily identified because female gametes (eggs) are easily visible as they are large and extruded onto the pleopods inside the brood chamber. Male gametes (sperm) however, are small and males often lack any clear external indication of sexual maturity. Allometric differences in secondary sexual characteristics are generally used to identify those individuals producing gametes from immature individuals. The most common of these characteristics is the abdomen (for females) and chelae size (for males) relative to carapace width (Claxton *et al*., 1994; Comeau and Conan, 1992; Conan and Comeau, 1986; Gao *et al*., 1994; Sainte-Marie and Hazel, 1992; Stevens *et al*., 1993; Watson, 1970).

Maturation in brachyurans can either occur over a single, pubertal moult where sexual dimorphism becomes distinct, or as a gradual change over several moults (Hartnoll, 1978; Hartnoll, 1982). Sudden changes over a single moult are represented by discontinuities in growth curves, as seen in that for male propodus height and carapace width in *Rhynochoplax coralicola* (Gao *et al*., 1994), *Halicarcinus innominatus* (Dunnington, 1999) and *H. varius* (Hosie, 2004), and for female abdomen width in *Bathynectes superbus* (Lewis, 1977) and *Corystes cassivelaunus* (Hartnoll, 1978). Sexual dimorphism in species without a pubertal moult occurs with a gradual increase in relative growth, such as in the major chela of male *Heterozius rotundifrons* (Thompson, 1999), chela length in *Plagusia dentipes* (Tsuchida and Watanabe, 1997), chela and abdomen width in *Ovalipes stephensoni* (Haefner, 1985), and in abdomen width of *Uca thayeri* (Negreiros-Fransozo *et al*., 2003) and *Callinectes ornatus* (Haefner, 1990). *Halicarcinus cookii* experiences a pubertal moult (McLay, 1988; Melrose, 1975) and therefore sexual dimorphism should theoretically develop most dramatically over this single moult. In females the pubertal moult occurs over a large size range (4.61-9.01 mm carapace width), over 50% of the maximum size range in the study, while male maturity was unidentifiable. Hosie (2004) found that in both male and female *H. varius*, the pubertal moult amounted to nearly 50% of the maximum size range (6.3-12.5 mm and 5.65-9.8 mm carapace width for males and female

respectively). Similarly, immature male *Rhynchoplax coralicola* were reported to almost cover the entire adult size range of ~3.5 mm CW of a maximum 3.8 mm (Gao *et al*., 1994).

The allometric growth rates of *Halicarcinus cookii* are typical of the Brachyura (Dunnington, 1999; Hartnoll, 1978; Hartnoll, 1982). Males showed a negative allometry in abdomen growth. The male abdomen changes little throughout development, remaining triangular and barely covering the ventral surface of the sternum. In contrast, two distinct phases of abdomen growth were seen in females. Immature females showed positive allometry in abdomen width, while after the pubertal moult abdomen width became isometric and changed in shape. In female *H. cookii*, the abdomen changes from being flat and only partially covering the sternum, to convex and completely covering the sternum, creating a brood chamber in which broods are incubated. These differences allow for easy identification of males and females as well as immature and mature females in the field. These patterns are seen in many hymenosomatid species such as *Rhynchoplax coralicola* (Gao *et al*., 1994), *Amarinus laevis* (Lucas and Hodgkin, 1970a), *Halicarcinus innominatus* (Dunnington, 1999) and *H. varius* (Hosie, 2004).

Chelae size (propodus length and height) was compared between the sexes in *H. cookii*. Males showed very strong positive allometry of both propodus length and height throughout development. This pattern is typical of brachyurans (Hartnoll, 1978; Hartnoll, 1982; Hartnoll, 1985) and was reported for the hymenosomatids *Rhynchoplax coralicola* (Gao *et al*., 1994), *Amarinus laevis* (Lucas and Hodgkin, 1970a), *Halicarcinus innominatus* (Dunnington, 1999) and *H. varius* (Hosie, 2004). Immature female *H. cookii* showed isometric growth in propodus length and mature females showed a weak negative allometry in propodus length. For propodus height, immature females showed weak negative allometry while mature females showed weak positive allometry. Although these results were significant, there was a more obvious difference in chelae growth between males and females. Regression lines indicate that male chelae size increases at a much higher rate than in females, resulting in males having obviously larger chelipeds than females, providing another characteristic with which to

distinguish between the sexes. In male *H. cookii*, there was no distinguishable difference in chelae size relative to carapace width, so male maturity was estimated at an arbitrary > 7 mm based on spermatophore production (Chapter 3).

Chelae morphology can also be used as an indication of male maturity in brachyurans. Changes in the size, shape, setation and dentition of the chelae over the maturity moult have been used to distinguish between immature and mature males of the Majidae (Claxton *et al*., 1994; Comeau and Conan, 1992; Hartnoll, 1965). Over the maturity moult, males of the hymenosomatid *A. laevis* develop pulvini (prominent sacs) between the fingers of the chelae (Lucas and Hodgkin, 1970a) similar sacs are seen in *Hemigrapsus sexdentatus* (McLay, 1988). Male *H. innominatus* and *H. varius* develop an extra tooth on the dactyl of the cheliped which becomes more pronounced as an indication of maturity (Lucas, 1980; McLay, 1988; Melrose, 1975; Menzies, 1988). Male *H. cookii*, however, show no distinct change in morphology of the chelipeds throughout development (Melrose, 1975). Therefore, neither sperm production nor chelae morphology can be used as reliable indications of male maturity in *H. cookii*.

Chelae size relative to body size is the most commonly used indication of male maturity in many species such as *Uca* spp. (Negreiros-Fransozo *et al*., 2003), *Heterozius rotundifrons* (Thompson, 1999) and *H. varius* (Hosie, 2004). However, in *Chionoecetes opilio*, morphologically immature males have been reported to produce sperm (Comeau and Conan, 1992). Lucas (1980) reported that in the hymenosomatids *Amarinus lacustris* and *A. paralacustris*, males classified by chelae size as immature were capable of copulating. Similarly, if sperm production defined male maturity in the hymenosomatid *Halicarcinus innominatus*, then males with 4 mm carapace width would be considered mature (Menzies, 1988). Dunnington (1999) suggested that the increase in chelae size may enable a mature male to copulate with a female more successfully due to the ability to physically restrain females. Morphologically immature majids, *C. opilio* and *C. bairdi*, were found to be unable to successfully restrain and therefore mate with, multiparous females in the wild (Claxton *et al*., 1994; Stevens *et al*., 1993, respectively). However, Paul (1992) reported that such males of *C. bairdi* could restrain soft, primiparous females long enough to copulate. If cheliped size is not an adaptation

primarily for restraining females, competition between males is a likely causal factor for the development of such weapons for purposes of fending off rival males (Andersson, 1994). In *H. cookii*, chelipeds are not used in displays, but they are used in agonistic encounters between males (Chapter 4). Males with larger chelipeds would logically have an advantage over those with smaller chelipeds, suggesting a selective force behind the development of large chelipeds in males, but not necessarily an indication of male maturity.

In hymenosomatids, the pubertal moult is also the terminal moult, after which growth no longer occurs. This cessation in growth also restricts the individual from shedding damaged or parasitized integument, and replacing lost limbs (Lucas, 1980). In the adult instar, the allometric relationship between body dimensions and the reference dimension is an allometry of size attained at the final moult, rather than growth rate (Hartnoll, 1982). A terminal, pubertal moult for both males and females is thought to occur in *Amarinus* and *Halicarcinus* species (Lucas, 1980). Such a terminal moult was obvious in female *H. innominatus* (Dunnington, 1999) and *H. varius* (Hosie, 2004). Similarly, a terminal, pubertal moult was obvious in female *H. cookii*, but there was little evidence suggesting a terminal moult in males. No large male *H. cookii* were observed to moult and the lack of a distinguishable difference in size or allometry between immature and mature male suggest that male *H. cookii* may not experience a terminal moult. However, if male *H. cookii* did not experience a terminal moult, they would be expected to grow much larger than females. As this was not observed, a terminal moult in males can be assumed to occur.

Moulting in crustaceans is controlled by hormones. Continued moulting after reaching maturity in brachyurans can be inhibited either by the excessive production of a moult-inhibiting hormone from the X-organ-sinus gland, or by the degradation of the Y-organ, which produces a moult-promoting hormone (Carlisle, 1957). Further investigations into the moulting patterns and controlling factors are required for a better understanding of growth and maturity in *H. cookii*.

The ability to easily separate individual *H. cookii* into sexes and level of maturity allowed relatively accurate sampling and analysis of the Kaikoura population. Hymenosomatids are characterized by short life spans (Lucas, 1980). Most appear to live approximately one year, such as *Amarinus laevis* (Lucas and Hodgkin, 1970b), *Halicarcinus innominatus* (Dunnington, 1999; Menzies, 1988) and *H. varius* (Hosie, 2004), while the circum-Antarctic *H. planatus* was reported to live up to three years (Richer de Forges, 1977). According to population size frequency data, the life span of *H. cookii* appears to be around 12-18 months with approximately 6 months as a mature instar. Although mean carapace widths remained relatively steady throughout the year (carapace width = 6.57-9.5 mm (males) and 6.74-9.25 mm (females)), there was a slight increase in mean carapace width in the summer months. The range of sizes also changed throughout the year, with more small individuals found during the winter months when immature individuals were in their highest proportion in the population. As summer approached the average size of immature females increased while their proportion in the population decreased, reaching a minimum from December 2004 to February 2005. Males were present in the smaller size classes throughout the year, including the summer months, and occupied the widest range of sizes.

There was no obvious discrete breeding or recruitment season in the *H. cookii* population as small (immature) individuals were present in each month. Ovigerous females were also found each month, suggesting that breeding and recruitment occurred throughout the year. Such year-round high percentages of ovigerous females were found in other hymenosomatid species such as *Hymenosoma orbiculare* (Broekhuysen, 1955), *Amarinus laevis* (Lucas and Hodgkin, 1970a), *Elamenopsis kempi* (Ali *et al*., 1995), *Halicarcinus innominatus* (Dunnington, 1999) and *H. varius* (Hosie, 2004). Peaks in the percentage of ovigerous *H. cookii* females found in the population occurred in the summer months. This summer peak was also seen in *A. laevis* (Lucas and Hodgkin, 1970a) and *E. kempi* (Ali *et al*., 1995). Such a peak was seen in the winter months in other species such as *Hymenosoma orbiculare* (Broekhuysen, 1955).

A clearly defined breeding season would be evidenced by synchronized egg development through the reproductive season. The majority of ovigerous females would therefore be expected to carry early stage broods at the beginning of the reproductive season, and later stage broods (closer to hatching) near the end of the reproductive season. This pattern was seen in *Hymenosoma orbiculare* (Broekhuysen, 1955) and *Elamenopsis kempi* (Ali *et al*., 1995). However, the *H. cookii* population showed no such pattern. The majority of ovigerous females carried early and late stage broods throughout the year. This lack of brood stage synchrony was also seen in *H. innominatus* (Dunnington, 1999) and *H. varius* (Hosie, 2004) and provides further evidence for a continuous breeding season.

Peaks in numbers of ovigerous females in the population can lead to peaks in recruitment. If there is a peak in the proportion of the population producing eggs and hatching larvae, recruitment is likely to peak slightly later, when the larvae have completed their planktonic life stage. The *H. cookii* population showed an increase in proportion of juveniles in the winter months suggesting an increase in recruitment some time before this period. These cycles of growth, reproduction and death are seen in other hymenosomatids such as *H. innominatus* (Menzies, 1988), *H. lacustris* (Walker, 1969), *H. varius* (Hosie, 2004) and *Rhynchoplax coralicola* (Gao *et al*., 1994). Brood development in *H. cookii* takes less than thirty days (Chapter 3) and planktonic larval development is likely to be similar to the 21-30 days recorded for *H. varius* at 16°C from hatching to the first juvenile crab instar (Horn and Harms, 1988). There is little information on the inter-moult periods or percentage moult increments (PMI) for *H. cookii* so an estimate of how long it takes for an individual to become large enough to be detected in the population will be tentative at best. By taking the mean percent moult increment of 18% and assuming this remained constant throughout the growth phase, it could be estimated that a female with 2 mm CW would moult approximately 10 times before reaching the mean female CW of 8.4 mm. According to Richer de Forges (1977), *Halicarcinus planatus* would take approximately 10 moults to grow to 2.6 mm CW with an average PMI of 15.4%. Although PMI tends to decrease as size increases, suggesting that the PMI of small individuals is likely to be higher (Richer de Forges, 1977), Hosie (2004) estimated that it would take at least 2-3 months before the crabs

reach a carapace width of 2.63 mm. As no first instars of *H. cookii* were found, and the smallest individuals were likely to be under-represented due to the crude sampling technique (the smallest found was 1.99 mm CW), the peak in recruitment is likely to lag close to a month after the peak in ovigerous females due to the duration of the planktonic larval phase.

The population sex ratio of all males to all females was observed to change throughout the study period. Changes in sex ratio may be the result of differing offshore movements between the sexes, uneven recruitment of sexes, or differential mortality (Leigh, 1970; Wenner, 1972). Males outnumbered females in only three months of the entire study period (June, August and October 2005). However, these results consider the entire population when only the operational sex ratio is reproductively important. When considering only sexually mature individuals, females consistently outnumbered males during the entire study period except one winter month (June 2005), and followed a similar trend to the population sex ratio during the peak breeding season of November 2004 to February 2005, suggesting that males were in short supply. During this period the operational sex ratio remained over 2 females per male, but by the winter months the sex ratio became more variable. This increase in variability may be due to a decrease in numbers of mature individuals due to a short life span. If reproduction competes more successfully for limited resources than survival, then both are likely to be put at risk (Calow, 1978). Therefore, as was suggested for *Amarinus laevis* (Lucas and Hodgkin, 1970a) and *Halicarcinus innominatus* (Dunnington, 1999), if mature female *H. cookii* are allocating more energy into reproduction than survival compared to males, their life-span may be shorter, explaining the decrease in operational sex ratio toward the end of the peak reproductive season.

Sex ratio as a function of size was also examined in *H. cookii*. The lack of significant difference in proportion of males and females in the smaller size classes (< 6 mm CW) suggest that both males and females recruit into the population at a ratio close to 1:1. However, females dominated the middle size classes (6-10 mm CW), while males were in significantly higher proportions in the larger size classes (10-14 mm CW). This is a common trend in the Brachyura. Female *Uca pugilator* consistently outnumbered

males, particularly in the 10-15 mm size class (Colby and Fonseca, 1984). Similarly, size distribution in *Aratus pisonii* was relatively normally distributed for females, but strongly skewed toward the larger size classes in males (Diaz and Eloy Conde, 1989). Wenner (1972) suggested several explanations for this pattern including differential mortality, differential migration or longevity, sex reversal and, the most likely scenario, differential growth rates. The higher proportion of females in the middle size classes is likely to be due to the terminal moult in this species. Growth ceases after the pubertal moult (at least in females, and assumed for males) so that females accumulate in these size classes, outnumbering males and do not grow larger. Males, however, grow beyond these middle size classes to dominate the larger size classes. Dunnington (1999) suggested this to be the case in the differing size class sex ratios of *H. innominatus*, and results from the study of *H. varius* by Hosie (2004) showed a similar pattern.

Sex ratios were further investigated by separating females into brood stages. The ratio of males to females carrying individual brood stages was highest during the spring-summer period (particularly October to November 2005). Stage 1 females were the only ones to ever outnumber males, and this only occurred during the summer months (November 2004, January to February 2005 and November to December 2005). Females carrying all other brood stages were outnumbered by males for the entire study period. The most limited females were those carrying no eggs at all, in fact, a mean of 96% of mature females were found to be carrying eggs throughout the study period. This is likely to be due to the short inter-brood period (see Chapter 3) after hatching and before laying the new brood, leading to a rarity of these females in the sample. Of the ovigerous females, those carrying broods at stages 3 and 4 were the fewest throughout the study period and, along with stages 2 and 5, were always found in smaller numbers than males. These differences are most likely to be due to the duration of incubation of individual brood stages. Females carrying broods at stages requiring the longest incubation time are likely to be found in higher numbers than those with a shorter incubation time. Therefore, stage 1 is likely to have the longest duration of approximately 44% of the total incubation time, whereas stages 3 and 4 are likely to have the shortest incubation time, comprising only 12% and 10% respectively.

Incubation duration is investigated further in Chapter 3. Differences in male behaviour toward females carrying different brood stages are discussed in Chapter 4 along with changes in male behaviour according to operational sex ratio.

The allometric growth patterns and population dynamics of *Halicarcinus cookii* are typical of Hymenosomatidae and the Brachyura. Males show positive allometric growth in chelae size and females show positive allometric growth in abdomen width from the penultimate instar to the mature instar. Chelae size and abdomen width are therefore distinct characteristics that can be used to easily distinguish between males and females. Growth ceases once maturation occurs (at least in females) causing an accumulation of mature females in the average size classes, while males tend to grow larger than females. Population dynamics of *H. cookii* are typical of many hymenosomatids. Although mature individuals are found throughout the year, *H. cookii* appears to have a short life span of approximately 12-18 months with only 6 months in the mature instar, resulting in a die-off during the winter months when recruitment is assumed to be high. Ovigerous females are found throughout the year, so the population showed no distinct breeding season, rather there was a peak breeding season during the summer months in which the highest numbers of individuals (mature females in particular) were found. Although mature females generally outnumbered males, the sex ratio became more varied during the winter months, most likely due to the disproportionate decrease in numbers of mature females compared to males. When separating females according to brood stage, females carrying all brood stages were limiting to males throughout the year except for stage 1 females who outnumbered males in 5 of the 15 months samples.

Chapter 3

Reproductive Biology

3.1 Introduction

Knowledge of the reproductive biology of any species is essential to understanding its population dynamics. Reproduction for most crustaceans involves the processes of copulation, fertilization, egg laying and incubation (Hartnoll, 1985). There have been many studies of the reproductive biology of commercially important brachyuran crabs such as *Chionoecetes opilio* (Beninger *et al*., 1988; Beninger *et al*., 1993; Comeau and Conan, 1992; Conan and Comeau, 1986; Elner and Beninger, 1995; Sainte-Marie, 1993), *C. bairdi* (Donaldson and Adams, 1989; Paul, 1984; Paul and Paul, 1992; Paul and Paul, 1996; Stevens *et al*., 1994) and *Cancer magister* (Hankin *et al*., 1989; Jensen *et al*., 1996; Swiney and Shirley, 2001) investigating breeding seasons, including sex ratios, ovary development and mating (Comeau and Conan, 1992; Swiney and Shirley, 2001), sperm supply and storage (Gardner and Williams, 2002; Jivoff, 1997a; Kendall *et al*., 2002; Sainte-Marie and Lovrich, 1994; Sainte-Marie *et al*., 2000), variability in incubation times (Jivoff, 2003b; Wear, 1974) and levels of fecundity (Hines, 1992; Jewett *et al*., 1985; Sainte-Marie, 1993; Sainte-Marie *et al*., 2002).

Halicarcinus cookii is an interesting subject for investigations into reproductive biology because it differs from the more commonly studied brachyuran species. In many brachyuran crabs, the moulting cycle is an important determinant of the timing of copulation (Hartnoll, 1969). For example, female *Cancer magister* (Hankin *et al*., 1989) and *Chionoecetes opilio* (Sainte-Marie *et al*., 2000) can only mate when their exoskeleton is soft immediately after moulting, resulting in discrete mating seasons in

these species. In contrast, *Halicarcinus cookii* shows little evidence of a discrete mating season, instead high proportions of ovigerous females producing successive broods occur throughout the year (see Chapter 2), suggesting that females can mate in a hard-shell condition after the pubertal moult.

Growth and reproduction can be considered competing processes in terms of the allocation of energy (Hartnoll, 1985). Decreasing growth can increase reproductive output (Hines, 1982a). The terminal moult in *H. cookii* allows females to produce broods continuously and successively, but the number of eggs per brood remains small. Small size and frequent production of small broods is characteristic of the hymenosomatid reproductive strategies (Melrose, 1975).

Being ovigerous throughout the year exposes *H. cookii* females to various environmental factors that can affect their reproductive output. Reproductive output is determined by the number of offspring produced over a life time (Shields, 1991), and this is determined by the number of broods produced, the number of eggs per brood, their incubation time and survival rates. As brachyuran eggs are carried externally under the female abdomen, species with discrete breeding seasons presumably release their larvae in optimal environmental conditions. The year-round brood production of *H. cookii* exposes the eggs to environmental extremes such as temperature, that may affect their incubation time or survival that a discrete breeding season would avoid (Jansen, 1974; Ottaway, 1976).

Crustacean sexual reproduction often involves the storage of spermatozoa by the female after copulation prior to fertilization and egg laying (Hartnoll, 1985). All brachyurans can store sperm for relatively long periods of time (often over successive moults, breeding cycles or even years), indicating that the sperm from one male is likely to encounter viable sperm from previous mates inside the females seminal receptacle, resulting in potentially high levels of sperm competition (Diesel, 1991). Sperm storage, viability and competition have been investigated in various species including *Metopograpsus messor* (Anilkumar *et al*., 1999), *C. opilio* (Beninger *et al*., 1988), *Menippe mercenaria* (Cheung, 1968) and *Callinectes sapidus* (Jivoff, 2003a).

Hymenosomatids store sperm in the spermathecae which are enlarged regions of the genital duct between the vagina and the oviduct where the spherical to ovoid spermatozoa are stored (Lucas, 1980).

Brachyuran spermathecae can be divided into two groups according to their position relative to the oviduct, which Diesel (1991) terms ‘dorsal-type’ and ‘ventral-type’, and this can have major implications for the fertilization process and hence the reproductive behaviour of males. Dorsal type spermathecae resemble enlarged tubes in which the oviduct opening is relatively dorsal and the vagina is positioned further away and more ventrally. This occurs in portunid crabs such as *Carcinus maenas* and *Callinectes sapidus* (Diesel, 1991). Ventral-type spermathecae are sac-like, with the oviduct and vagina situated ventrally and close together. This type occurs in spider crabs such as *Chionoecetes opilio* (Beninger *et al*., 1988) and *Inachus phalangium* (Diesel, 1989), as well as in the families Calappidae, Geryonidae, Leucosiidae and Corystidae (Diesel, 1991). The proximity of the oviduct to the vagina influence which sperm is used to fertilize the eggs. Species with dorsal-type spermathecae tend to use the oldest sperm (sperm from the first male to mate) first, whereas species with ventral-type spermathecae use the sperm closest to the entrance (sperm from the last male to mate) to fertilize the eggs first (Diesel, 1991).

Hymenosomatids are able to store sperm and fertilize many broods from a single copulation. Lucas (1980) reported that *Amarinus laevis* and *H. ovatus* produced viable egg masses without re-mating, and a hybrid of *Amarinus lacustris* and *A. paralacustris* produced a succession of fifteen viable broods with sperm from a single copulation. This suggests that opportunities to encounter mates or re-mate may be low and that there is a potential for male-male competition (including sperm competition) which may influence male behaviour and lead to greater dimorphism of secondary sexual characters.

The terminal/pubertal moult of *Halicarcinus cookii* implies that individuals are able to produce offspring continuously, but the number of offspring produced per brood is limited due to small female body size. This Chapter will be the first extensive

investigation into the reproductive biology of *H. cookii*. The morphology of the reproductive structures will be examined and the potential implications of these are discussed. The most prominent factors when considering reproductive biology of crustaceans will also be examined, including relationships between female size and brood size and female size and gonad size, embryo size and fecundity, sperm transfer and storage, as well as the effect of temperature on embryo development

3.2 Methods:

Reproductive Structures

To investigate morphological differences between adult and juvenile males and females, the first pleopods of adult and juvenile males and adult and juvenile females and the sternum of adult and juvenile females were viewed using a scanning electron microscope. All specimens were stored in 80% ethanol prior to preparation. Each specimen was then freeze dried by plunging it into liquid nitrogen. The tips of the pleopods were compared between adult and juvenile males, and the structure of both the endopod and exopod of the pleopods were documented including how the eggs are attached to the female pleopod. Pleopods and gonopores of adult and juvenile females were also compared.

110 female *Halicarcinus cookii* were selected for dissection to investigate the structure and proportion of body weight of the female reproductive organs. Females were either killed by freezing or selected shortly after death and blotted dry with a paper towel before dissection. All weights were obtained using a Sartorius TE153S scale (accurate to 0.001 g). Measurements of the carapace width (CW) of each female were taken using a Mitutoyo™ digital calliper (accurate to 0.01 mm). The initial weight of each crab was recorded. The eggs were then removed from the pleopods and their stage of development was recorded as 0, 1, 2, 3, 4 or 5 (for a description of egg stages see Table 3.1 (modified from Menzies (1988), Dunnington (1999) and Hosie (2004)). To compare individual body weight with and without eggs, crabs were weighed again after the eggs were removed. The eggs were collected and counted to investigate fecundity.

Gono-somatic index

Dead female *H. cookii* were placed in a Petri dish underneath a dissection microscope. Access to the gonads was gained by making and incision into the dorsal margin of the carapace and continuing around the edge until the entire carapace could be removed. After removing the cardiac stomach and heart, the gonads were easily accessible. Gonads were then removed and placed on a glass slide, where excess water was blotted with a paper towel, and weighed to calculate the gono-somatic index (GSI). GSI

was calculated as the ratio between gonad wet weight (GW) and wet body weight (BW) using the formula:

$$GSI = GW/BW \times 100$$

The mean GSI was calculated for each brood stage (n = 13, 19, 25, 16, 18 and 19 for stages 0-5 respectively) and results were compared using a one way ANOVA. Prior to each analysis, variances were tested for homogeneity using Cochran's test.

Egg Size

Throughout the Chapter, 'egg' refers to the embryos carried inside the female brood chamber rather than the unlaid eggs in the ovary. To investigate differences in egg size at different stages of development, the diameter of 20 eggs at each stage of development (collected from different females) were measured with an eyepiece micrometer (accurate to 0.03 mm). Eggs of *H. cookii* were considered to be spherical, so volume was calculated using the formula:

$$V = 4/3 \times (\pi \times r^3)$$

Mean egg volumes for each stage of development were calculated and compared using a one-way ANOVA. Differences between means were then compared using Tukey's HSD test.

Sperma-somatic index

Prior to each dissection, spermatheca fullness was visually estimated through the transparent sternum in the same manner described in Chapter 2 (0, 10, 25, 50, 75 or 100%). After gonads had been removed and weighed, spermathecae were easily visible from the dorsal view of the dissected crab. The spermathecae were then lifted with tweezers, with care not to puncture the soft tissue, placed on a slide and weighed. Sperma-somatic index (SSI) was calculated to produce a percentage of total female weight allocated to spermathecae using the formula:

$$SSI = SW/BW \times 100$$

Where BW = female wet body weight and SW = spermathecae wet weight.

Sperm supply

During monthly population surveys, the spermathecae fullness for each mature female was recorded to produce a measure of mating frequency over the 12 month period. Spermathecae fullness was estimated visually by looking through the transparent sternum of the female when the brood chamber was held open. Estimates of fullness were recorded as 0, 10, 25, 50, 75 or 100% (see plates 3.1-6).

To investigate the number of times a female must mate to fill the spermathecae, 20 females were selected and their carapace measured. A digital photograph of the spermathecae was taken through a dissection microscope and then the female was placed with a male of known carapace width in a 2 L ice cream container of seawater and monitored for mating. If mating did not occur, the male was replaced and the new pair was monitored again. This occurred until there was a successful mating, after which a second digital photograph of the spermathecae was taken. Photographs were analysed and compared using ImageJ 1.33u. Size scales were set as evenly as possible for all photographs of a single female to ensure the most accurate comparison between photographs was made. Each individual spermatheca was outlined using a freehand selection option and the area was calculated. The area was outlined and measured at least ten times to allow for variance in freehand drawing. The mean area was added to that of the opposite spermatheca to produce an estimate of the area encompassed by the spermathecae. The percentage increase in area over successive photographs was produced using the formula:

$$\% = (A/B-1) \times 100$$

where B is the estimate of spermathecae area before the copulation and A is the estimate of spermathecae area after the copulation in question.

Twenty females (based on availability), with a range of spermathecae fullness, but most with spermathecae estimated to be 100% full, were selected and kept for 24 h with a selection of males (n ~ 10) to provide opportunity to fill their spermathecae. The females were then isolated from males and kept in a separate tank for the remainder of the experiment. Females were tagged with commercial bee tags attached with super glue to their carapaces. This allowed individual crabs to be monitored through each brood cycle. Every 3 or 4 days the brood stage of each crab was recorded. Eggs were not removed and counted in an attempt to avoid the extremely high mortality, potentially due to the stress of egg removal, experienced by Hosie (2003).

At the onset of the experiment, a digital photograph of the spermathecae of each individual female was taken through a dissection microscope. After each female had laid a new brood (was observed to progress from stage 5 to stage 1), another digital photograph of the spermathecae was taken through a dissection microscope to compare to the photographs taken during previous broods. This allowed a direct photographic comparison of sperm use. Photographs were compared using ImageJ 1.33u as described above. Percentage decrease in area over successive photographs was calculated using the formula described above. A control experiment was also conducted for comparison, using the methods described above, but with a male present in the holding tanks.

Sperm Storage

To investigate the possibility of sperm mixing and sperm priority in the spermathecae, 12 male crabs of various sizes were selected for sterilization. Each male was labelled with a commercial bee tag attached with super glue.

These males were transported to the Christchurch Hospital Oncology Department for sterilization through exposure to gamma radiation. All 12 crabs were irradiated together to 40 Gy, using 6 MV photons from a Varian 2100C/D linear accelerator (LINAC). The crabs were irradiated in an 8 cm depth of sea water using two parallel opposed beams: with the crab container setup in the middle at the isocentre (the point the LINAC rotates around). Half the dose was delivered from above and half from below to ensure a

uniform dose distribution throughout the whole container of water. The crabs were irradiated while immersed in sea water to ensure charge particle equilibrium and full scatter. The LINAC was calibrated to deliver 1cGy per MU (monitor unit) to the point 100 cm from the source of the radiation conditions (isocentre) at a depth of 1.5cm in water for 6 MV photons (a point called dmax, which is the point that would receive the maximum dose from a beam).

The amount of MU required to deliver a dose of 40 Gy to the whole volume of water was calculated to be 1960 MU delivered from each beam using measured dose data based on the set-up conditions: A 25 x 25 cm field was used which was large enough to completely cover the whole container of crabs, a total depth of 8 cm of water was used, and this was centered at the isocentre. The 40 Gy was prescribed to the middle of the container (at the isocentre and 4cm depth in water).

Once irradiation was complete, the crabs were transported back to the laboratory where they were allowed to mate with females. As crab sperm are immotile, spermatozoa from irradiated males were assumed to have equal opportunity with those from healthy males to reach, but would fail to fertilize an egg, thus producing an unfertilized brood despite the successful sperm storage.

Thirty females with little or no sperm stored in their spermathecae were selected and each female was also labelled with a commercial bee tag for ease of monitoring. The females were then separated into three groups of differing mating sequences: Group A: Females who only mated with an irradiated male (control), B: Females who mated with an irradiated male followed by a healthy male, and C: Females who mated with a healthy male followed by an irradiated male.

Females were then monitored until they produced their next brood of eggs. Soon after a brood was laid, it was carefully removed from the pleopods, counted and the proportion of unfertilized eggs recorded. During the dissections of females described above, the number of unfertilized eggs was recorded for 10 females to control for females who have only mated with healthy males. However, due to the high level of

mortality caused by either stress or fatal injury during egg removal, this approach was abandoned for a simple observation of broods produced.

The eggs were initially allowed to begin developing before examination. Unfortunately, unfertilized eggs were lost from the pleopods within the first few days after being laid. However, there was a difference in colour between fertilized and unfertilized eggs. Unfertilized eggs were a much paler and duller orange compared to healthy, fertilized eggs. This was then used as the determining factor to distinguish between eggs. Any mixing of sperm would be indicated by only a proportion of eggs remaining and developing in a single brood.

Incubation Time

The effect of temperature on timing of brood development was investigated for possible extrapolation to differences in timing of brood development in the field during different seasons. During January 2005 - March 2005, four temperature rooms were set up to remain at relatively constant temperatures of 5, 10, 15 and 20^{o}C. For each temperature, 20 females, each marked with a commercial bee tag bearing a different number, were placed into two 50 × 20 × 5 cm trays with 3 L seawater and a fish tank air bubbler. A male was placed in each tray to ensure a constant sperm supply. Each day the water temperature and brood stage for each female was recorded and the water replaced.

Females were monitored through one complete brood cycle. The beginning of the cycle was recorded from the first change in brood stage observed and ended when that same stage was reached in the following brood so ensure an accurate record of the beginning of a stage. For example, if the female was first observed to carry a brood at stage 2, the recorded brood cycle began only when the brood first developed into stage 3, and ended the day before the first observation of stage 3 of the following brood.

The mean duration of each brood stage at each temperature was calculated and significant results were compared using Tukey's HSD test. The inter-brood period was

also recorded and compared between temperatures to investigate the influence of temperature on the time it takes for a female to lay a new brood.

Pre-pubescent maturity

An investigation into the timing of gonad maturity was conducted to determine if sexual maturity was determined strictly by the pubertal moult or whether there was evidence of pre-pubescent maturity.

30 males ranging in carapace width from 4.41 mm to 11.42 mm were dissected and examined for the presence of spermatozoa in the testes and/or vas deferens. Due to the inconclusive results of a possible indication of male maturity, such as the ratio between CW and propodus height described in Chapter 2, the presence of spermatozoa would also potentially provide an indication of male sexual maturity. This could be related to an average carapace width that could be used in population surveys to roughly distinguish between juvenile and adult males.

Nineteen juvenile females were observed through their pubertal moult after being isolated from any males. The females were monitored after their pubertal moult for their first brood of eggs. If the female produced a brood within 3 days, this was considered an indication of pre-pubescent gonad maturity. The females were then monitored to see if the eggs were fertilized. Eggs were considered fertilized if they developed into stage 2 eggs. If the eggs remained at stage 1 and were eventually lost from the brood chamber, they were considered unfertilized. Fertilized eggs were considered an indication that precocial mating (females being capable of successfully mating prior to the pubertal moult) and trans-moult sperm retention occurs in *H. cookii*.

Table 3.1 Egg descriptions of the 6 brood stages of *Halicarcinus cookii.*

Brood Stage	Description
0	No brood Empty chamber
1	Yolk bright orange Little or no cleavage in the egg
2	Yolk orange Obvious cleavage (clear area inside membrane)
3	Orange yolk with development of chromatophores (black pigmentation) Obvious cleavage, about 50% of yolk remains
4	Orange yolk with chromatophores and the development of eyespots Obvious cleavage, about 25% of yolk remains
5	<10% of orange yolk remaining, eyespots prominent, Larvae are fully developed and close to hatching

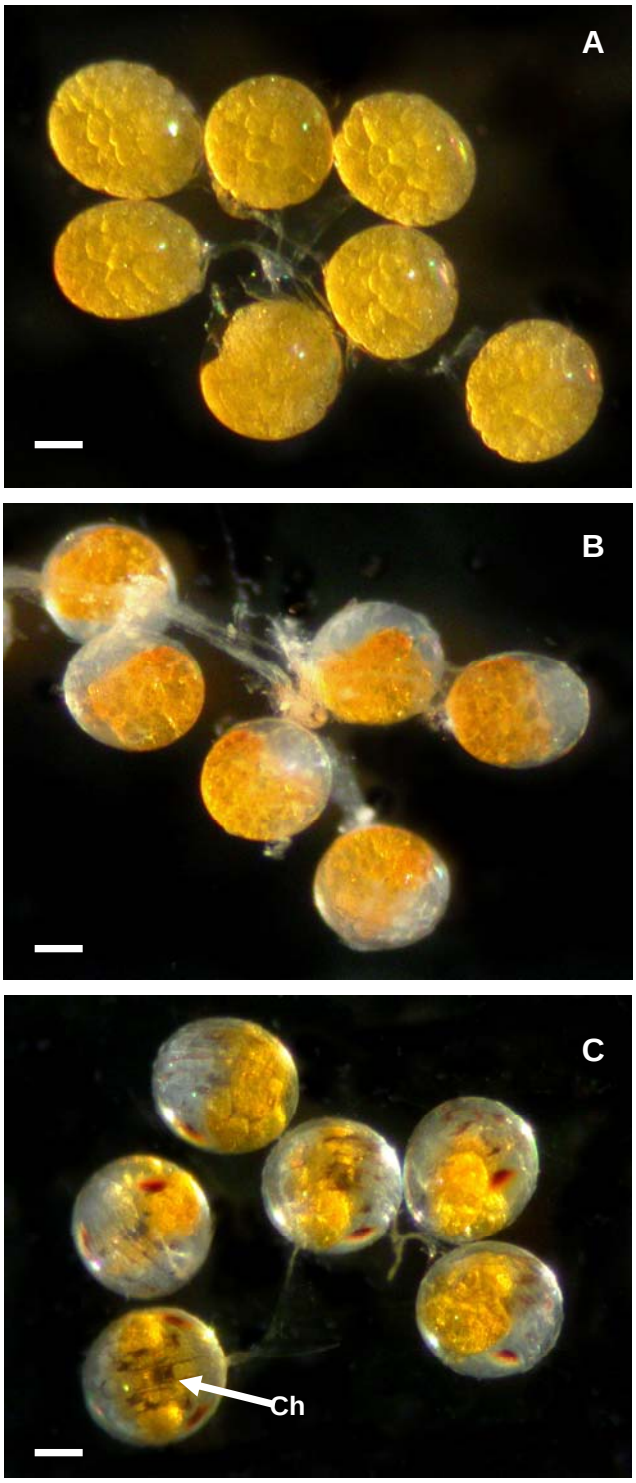


Plate 1.1 Embryos of *Halicarcinus cookii* at each stage of development. (A) stage 1, (B) stage 2, (C) stage 3, (D) stage 4, and (E) stage 5. Arrows indicate chromatophores (Ch) and eye spots (e). Scale bar ~ 0.01 mm. See Table 3.1 for descriptions of brood stages.

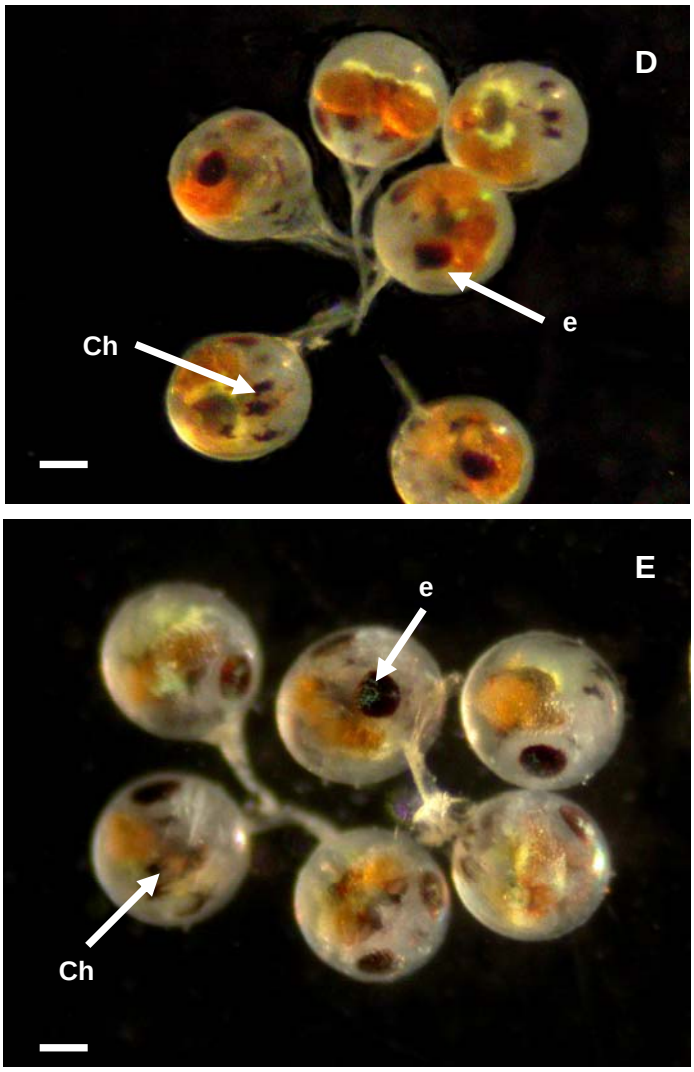


Plate 3.1 Continued. Embryos of *Halicarcinus cookii* at each stage of development. (A) stage 1, (B) stage 2, (C) stage 3, (D) stage 4, and (E) stage 5. Arrows indicate chromatophores (Ch) and eye spots (e). Scale bar = 0.01 mm. See Table 3.1 for descriptions of brood stages.

3.3 Results

Reproductive Structures

There was little morphological difference between adult and juvenile male pleopods (plates 3.2 and 3.3). However, the fold of exoskeleton appeared to stretch further around in the adult pleopod than the juvenile pleopod, forming a more complete tube in the adult rather than an open groove in the juvenile. Sensory setae and residual sperm were also visible at the very tip of some adult male pleopods, which was absent in the juveniles.

There were long setae on the adult female exopod while eggs were attached to the endopod, both of which were absent in the juvenile (plates 3.4 and 3.5). Individual eggs were attached to long filaments protruding from the endopod in groups of three with an extension of the egg membrane (Plate 3.6). The setae on the adult female exopod were covered in debris while the eggs and endopod were relatively clean. Since hymenosomatids have terminal ecdysis they cannot use a moult to clean the pleopods. The juvenile exopod was larger and flatter than the endopod but had not developed setae.

There was no operculum or similar structures observed covering the gonopores, suggesting they were permanently open. The gonopores of the juvenile female appeared to be only small slits in the sternum, whereas the adult gonopores were larger and more widely open (plate 3.7).

Spermathecae were ventral-type with the oviduct and vaginal opening positioned close together. Eggs pass through the oviduct into the spermathecae where they are fertilized by the sperm closest to the vaginal opening, through which they are then laid and attached to the pleopods to develop until hatching into zoea (plate 3.8).

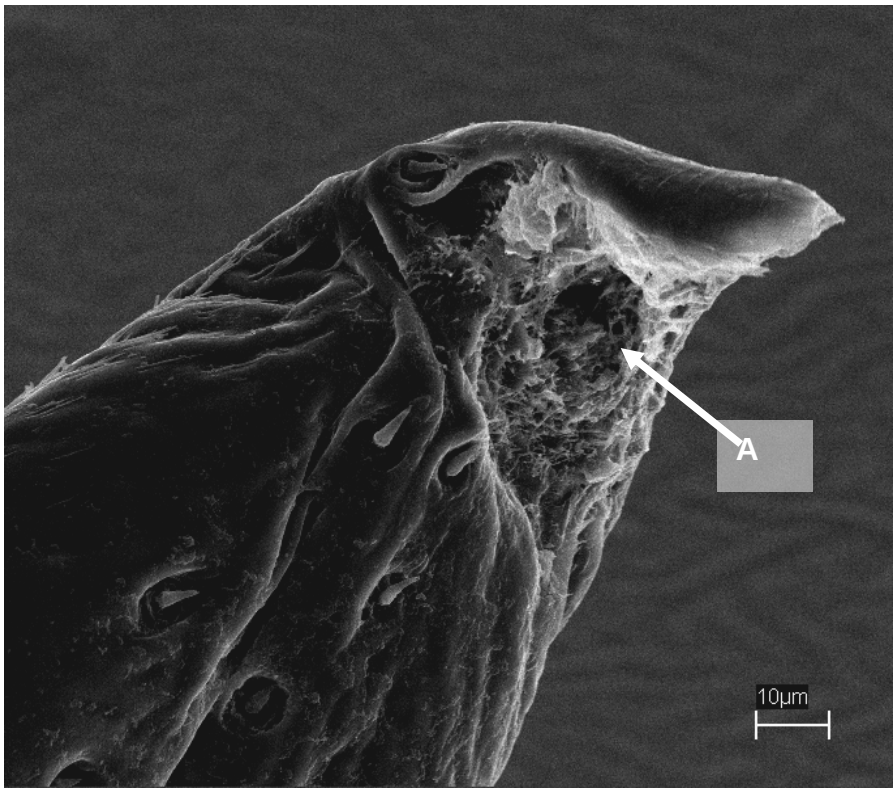


Plate 3.2 Scanning electron microscopy photograph of an adult male *H. cookii* gonopod. **(A)** shows residual sperm.

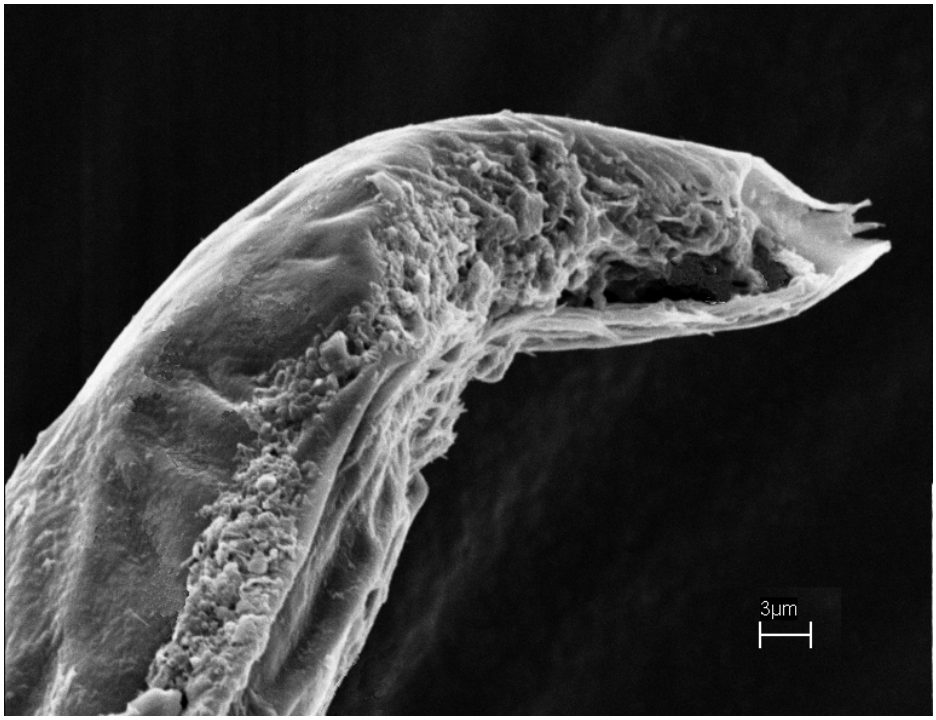


Plate 3.3 Scanning electron microscopy photograph of a juvenile male *H. cookii* gonopod.

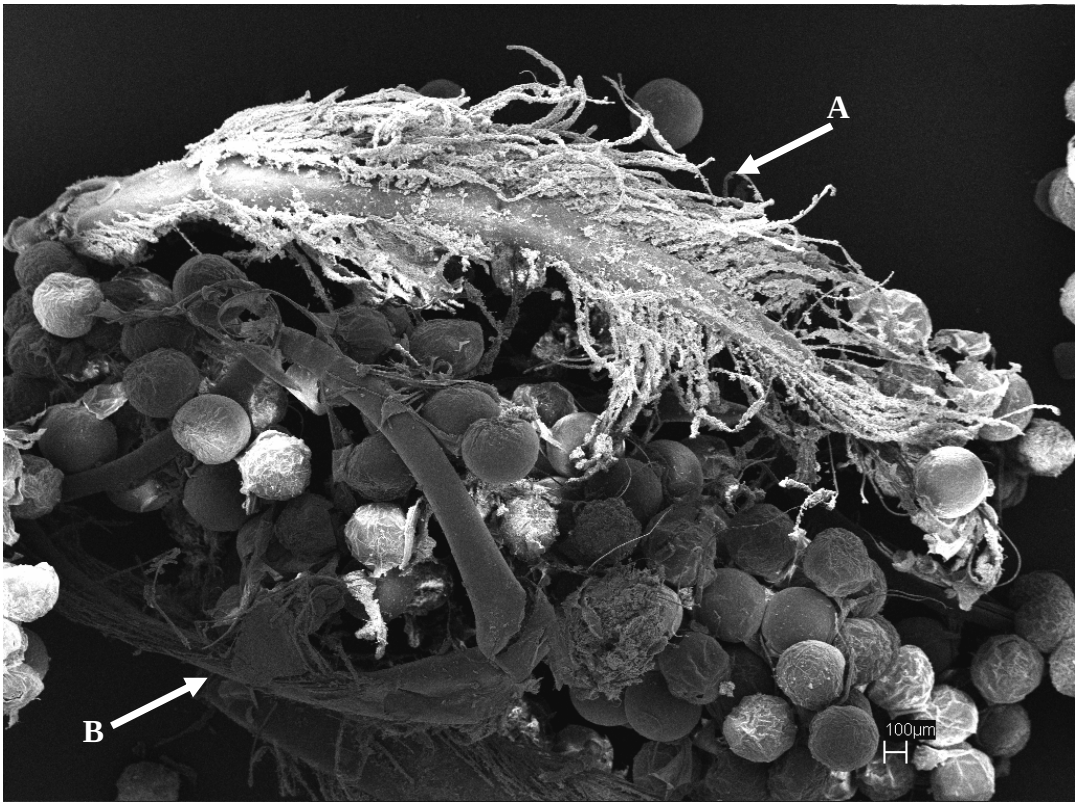


Plate 3.4 Scanning electron microscopy photograph of an adult female *H. cookii* pleopod showing cleaning setae on the exopod **(A)** and the eggs attached to the endopod **(B)**.

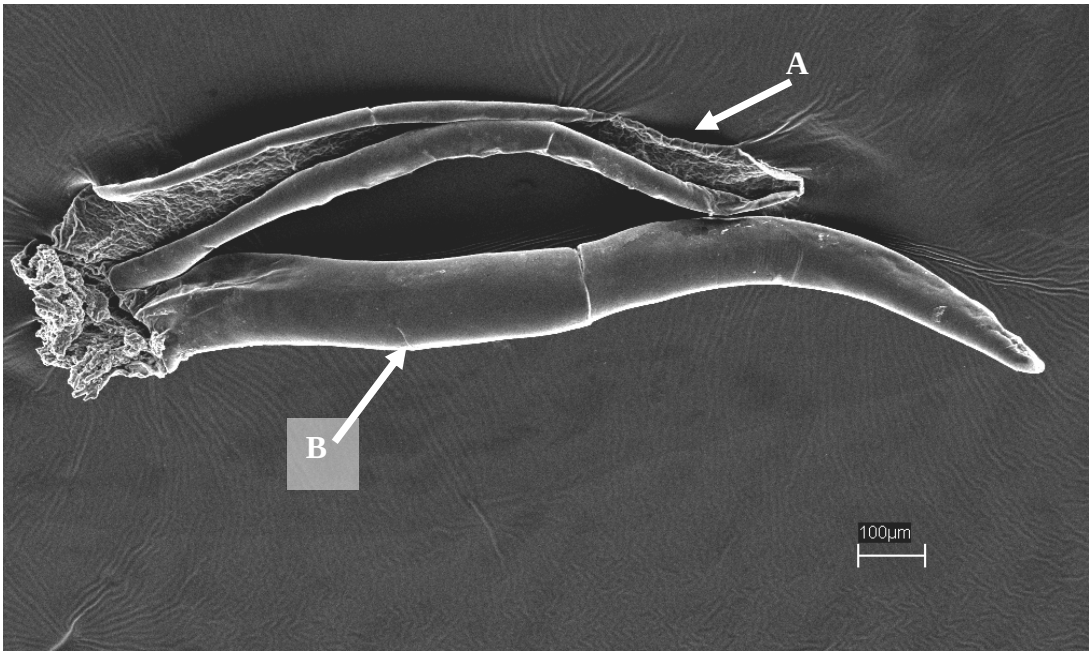


Plate 3.5 Scanning electron microscopy photograph of a juvenile female *H. cookii* pleopod, showing the exopod with undeveloped setae **(A)** and the endopod **(B)**.

A

B

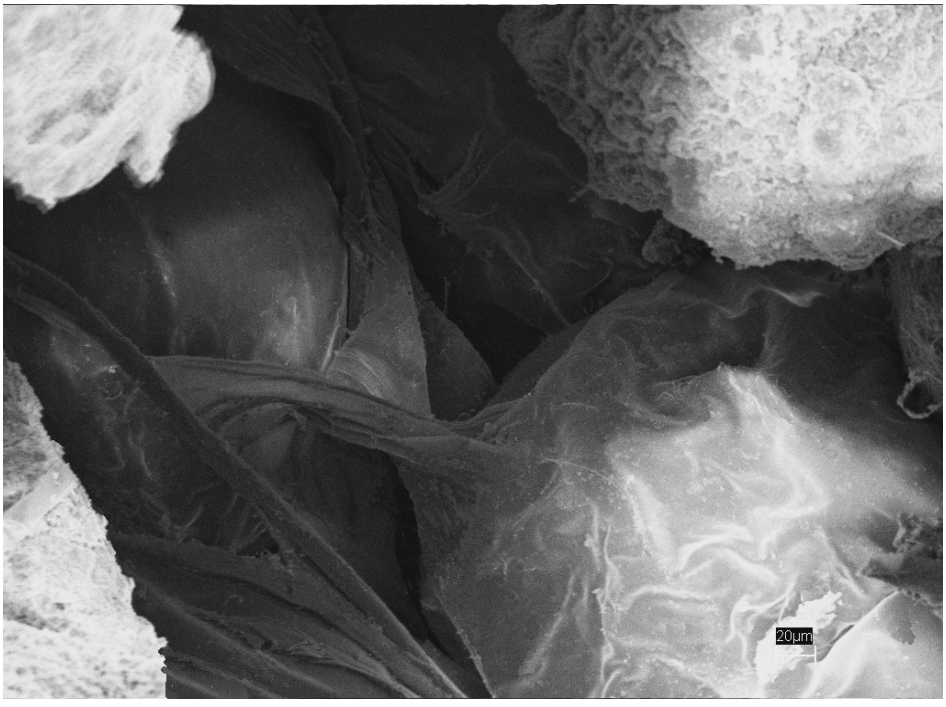


Plate 3.6 Attachment of eggs to the adult female pleopod. **(A)** Filaments, to which eggs are attached, protruding from the pleopod in groups of three, **(B)** egg attached to each filament with an extension of the egg membrane.

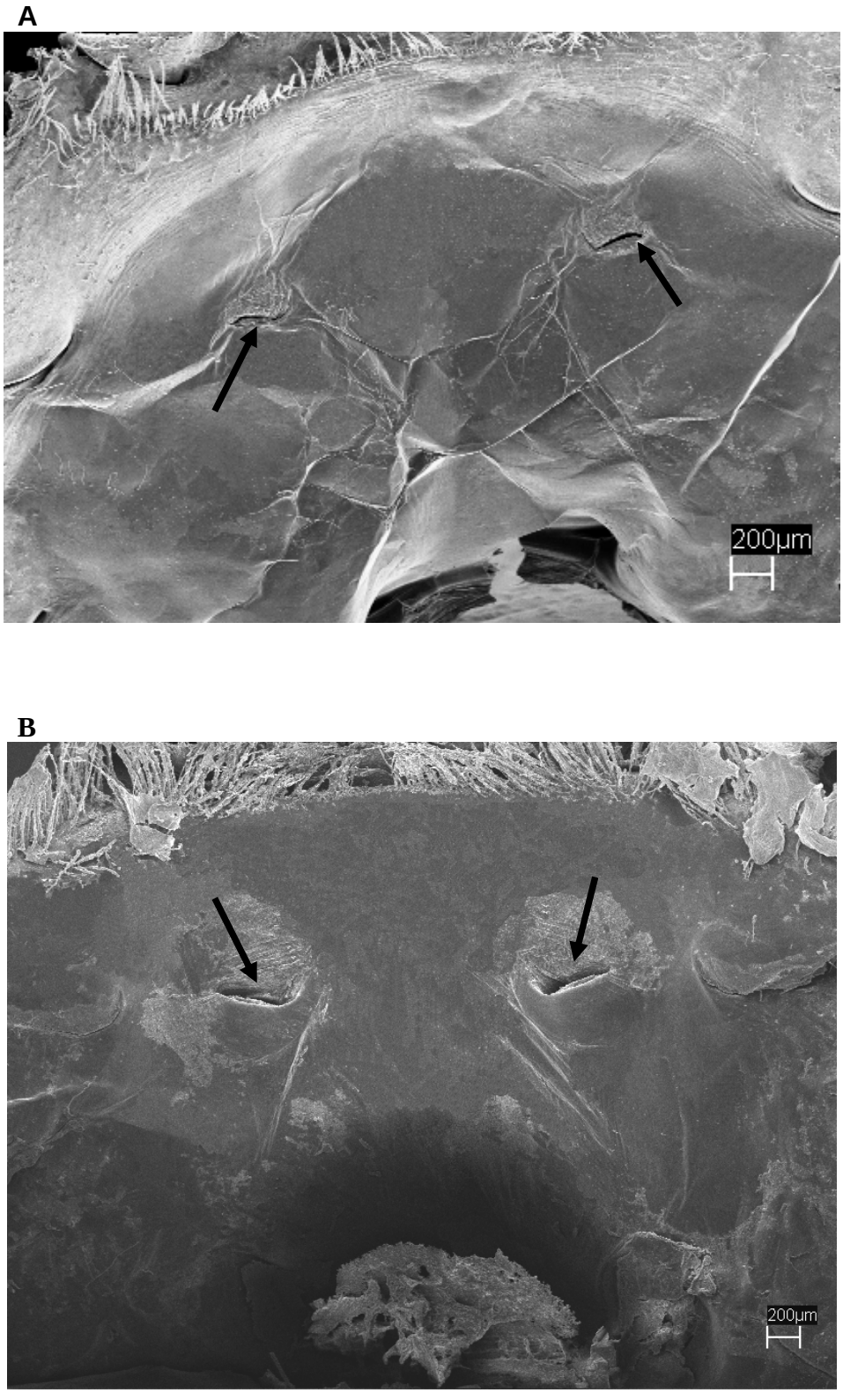


Plate 3.7 Scanning electron microscopy photograph of the female *H. cookii* sternum and gonopores (indicated by arrows) of **(A)** a juvenile in the penultimate instar and **(B)** an adult.

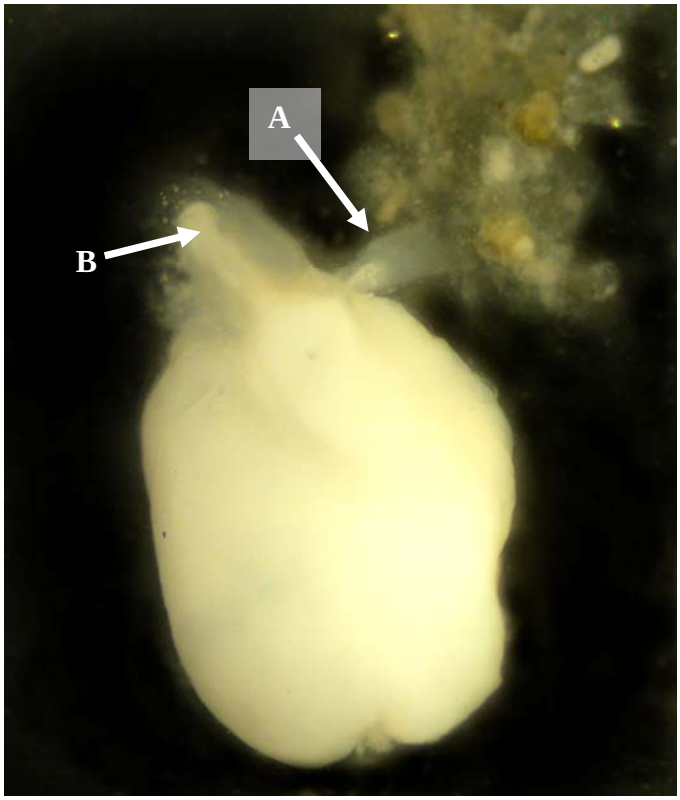


Plate 3.8 A single spermatheca dissected from a female *H. cookii*. Note the oviduct, **(A),** through which eggs pass into the spermathecae and are laid out of the vaginal opening at **(B)**.

Gono-somatic index

A difference in ovary mass between females carrying different brood stages was easily observed during dissections. The ovaries of females carrying early stage eggs were lighter green in colour and were much smaller than those of females carrying later stage eggs. There was a general trend of increasing gonad weight with more developed brood stage (Figure 3.1). There was a significant difference in gono-somatic index (GSI) between females carrying broods at different stages ($F_{5,122}$= 60.08, $p < 0.001$). Further analysis with a Tukey's HSD test for unequal N revealed that stages 0, 1 and 2 were not significantly different, and stages 3, 4 and 5 were not significantly different. Stage 1 females had the lowest mean GSI (2.22 ± 0.26) and stage 5 females had the highest mean GSI (10.19 ± 0.68). Female gonads therefore develop concurrently with broods and are at their maximum weight (maturity) when the fertilized broods are closest to hatching.

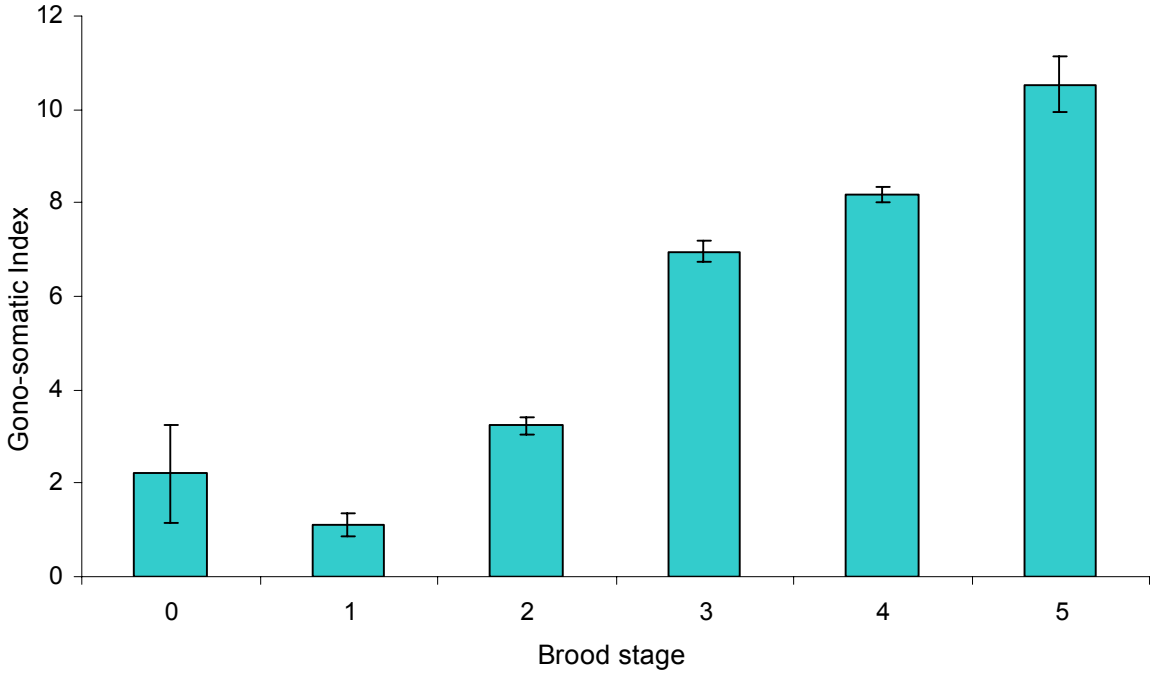


Figure 3.1 Mean gono-somatic index (±1 S.E.) of female *Halicarcinus cookii* according to brood stage (0-5), n = 12 (stage 0), 15 (stages 1-3), 16 (stage 4) and 17 (stage 5).

Egg Size

Mean egg diameters were calculated and compared between the five brood stages, stage 1: 0.363 mm, stage 2: 0.394 mm, stage 3: 0.422 mm, stage 4: 0.437 and stage 5: 0.452 mm. Mean volumes were subsequently calculated as: stage 1: 0.025µl (mm^3), stage 2: 0.032µl, stage 3: 0.039µl, stage 4: 0.044µl and stage 5: 0.049µl. From stage 1 to stage 5, the eggs showed a mean 92% increase in volume.

There was a significant difference in egg volume between stages of development ($F_{4, 95}$ = 144.64, p <0.001). Further analysis using Tukey's HSD test showed that all brood stages were significantly different from each other (p < 0.01 in all cases).

Fecundity

There was no significant difference in the number of eggs per clutch according to carapace width (CW) between stages 1 and 5 (ANCOVA $F_{1, 36}$ =1.51, p > 0.05) indicating that there was negligible egg mortality during development. Therefore data of brood sizes were combined for each stage of development for further analysis.

Brood size ranged from 306 (body weight (BW) = 0.196 g, carapace width (CW) = 5.47 mm) to 2438 (BW= 0.698 g, CW= 11.43 mm). Body weight (after removal of the brood) and CW showed a positive relationship described by the equation:

$$BW = 0.0071 \times CW^{1.6917}$$

With the R^2 value of 0.61 a rough estimate can be made that a female with average carapace width of 8.40 mm would have a body weight (excluding eggs) of approximately 0.260g and carry about 1146 eggs per brood.

There was a general trend showing that brood size increased with greater female CW. The R^2 value indicates that female CW accounted for 69% of the variation in clutch size (Figure 3.2).

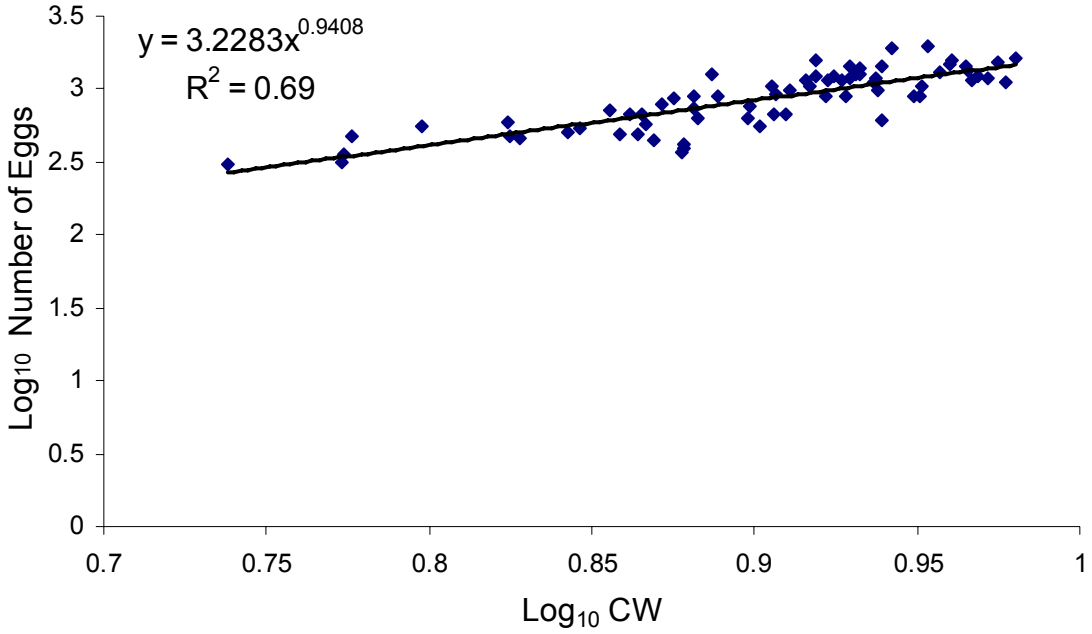


Figure 3.2 Relationship between $\log_{10}$ number of eggs and $\log_{10}$ female carapace width, n = 86. Regression equation and R^2 value are shown.

Sperm supply

Throughout the population survey discussed in Chapter 2, a visual estimate of spermathecae fullness was made for every adult female found. This provided an indication of when most mating was occurring and potentially how long the females stored sperm in their spermathecae. There was an increase in visual estimate of spermathecae fullness toward the end of the warmer months. The minimum estimate of spermathecae fullness was found in October 2004 and the maximum estimate was recorded in March 2005. Although spermathecae fullness showed a similar trend to male numbers, peaking in summer and dropping to a minimum in winter, the extremes lagged several months behind that of the number of mature males (Figure 3.3). As female numbers follow a similar trend to male numbers throughout the year (Chapter 2), this suggests that the majority of females mature in early summer and take 2-3 months to fill their spermathecae while the number of males, and therefore sperm supply peaks.

Sperma-somatic index

There was a significant difference in SSI for each level of spermathecae fullness visual estimate ($F_{5, 90}$= 77.47, p<0.001). Table 3.2 shows the mean SSI for each level of fullness and those that were not significantly different from each other. Females with the highest SSI had a visual estimate of 100% spermathecae fullness, while the lowest SSI was in females with a visual estimate of 0% fullness, but mean SSI overlapped in all homogenous groups.

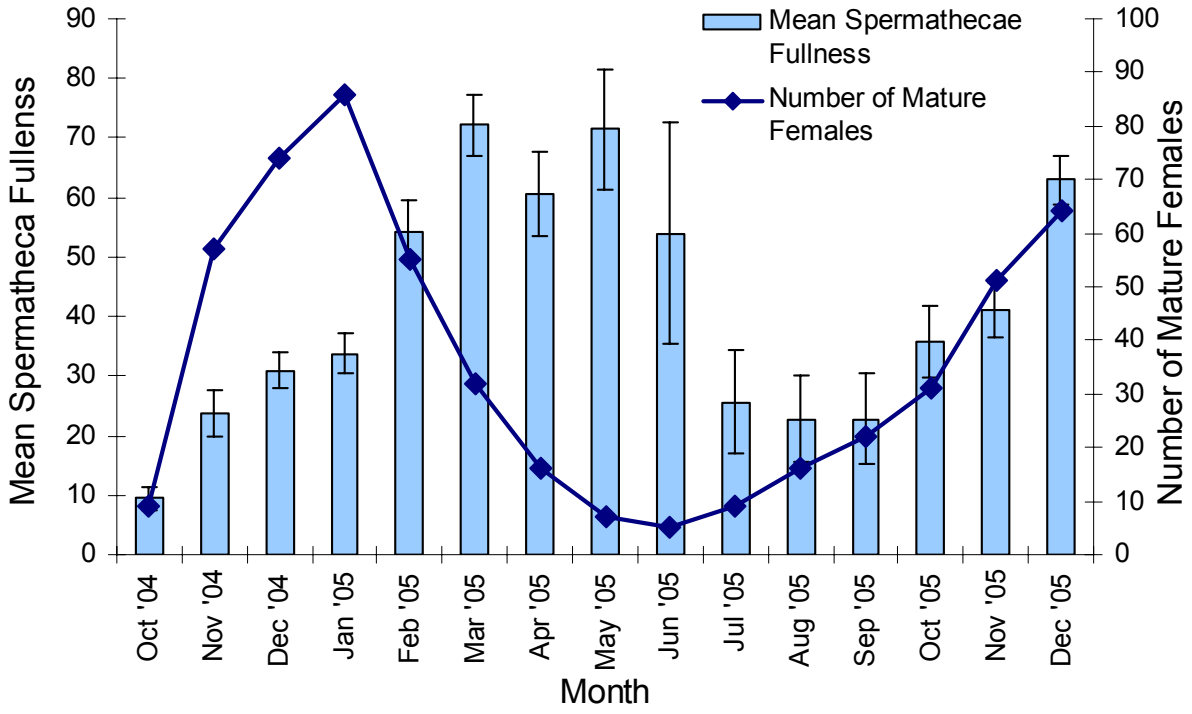


Figure 3.3 Comparison of the number of mature males found throughout the year (see Chapter 2) and the estimated spermatheca fullness from each monthly population sample. Numbers indicate number of mature females found each month.

Table 3.2 Mean sperma-somatic index for the visual estimates of spermatheca fullness. Letters indicate homogenous means (Tukeys HSD test p > 0.05), n = 10 for all groups.

Visual Estimate	Mean SSI	Standard Error
0%	0.04	0.01
10%	1.31[a]	0.1
25%	2.03[a]	0.23
50%	1.62[a]	0.19
75%	2.31[a,b]	0.3
100%	3.38[b]	0.43

Sperm supply

Analysis of spermathecae photographs before and after the female had copulated allowed for an estimate of the amount of sperm transferred and the number of copulations required to fill the spermathecae. However, images analysed were only 2-dimentional and did not allow for a 3-dimentional increase in size.

The spermathecae increased from a visual estimate of 0%-100% after only three or four copulations (plate 3.9). There was an average of 37.7% (S.E = 0.49) increase in spermathecae surface area after each copulation. Percentage increase of spermathecae surface area ranged from 9.3% (as the female's third copulation with a male with carapace width of 8.4 mm), to 73.44% (as the female's second copulation with a male of carapace width of 10.2 mm). Percentage increase was also positively correlated to carapace width of the male (Figure 3.4).

Although crabs in the sperm use experiment were only checked every three or four days and eggs were not removed, there was high mortality of females. This was most likely due to the stress caused by the experiment, or simply due to the fact that the experiment was conducted at the end of the summer season and the females were potentially facing the natural end of their life span. Out of the original 20 females, only three survived to produce a third brood. Nevertheless, photographs of spermathecae were still analysed, including those of females only producing a second brood and results could be somewhat reliable due to the range of spermathecae fullness prior to the experiment. There was a mean of -22.7% (S.E = 0.12) change in visible spermathecae surface area after the production of a new brood, ranging from -11.3% to -41.0%.

Females in the control treatment had a negligible mean change in spermathecae surface area of 7.51%. However, 50% of these females increased in spermathecae surface area over successive broods with a mean increase of 24.32%. The remaining 50% decreased in spermathecae surface area by a mean of -9.31%. There was a significant difference in percent change in spermathecae surface area between the control and the experiment ($F_{1,26}$= 23.5, $p < 0.001$).

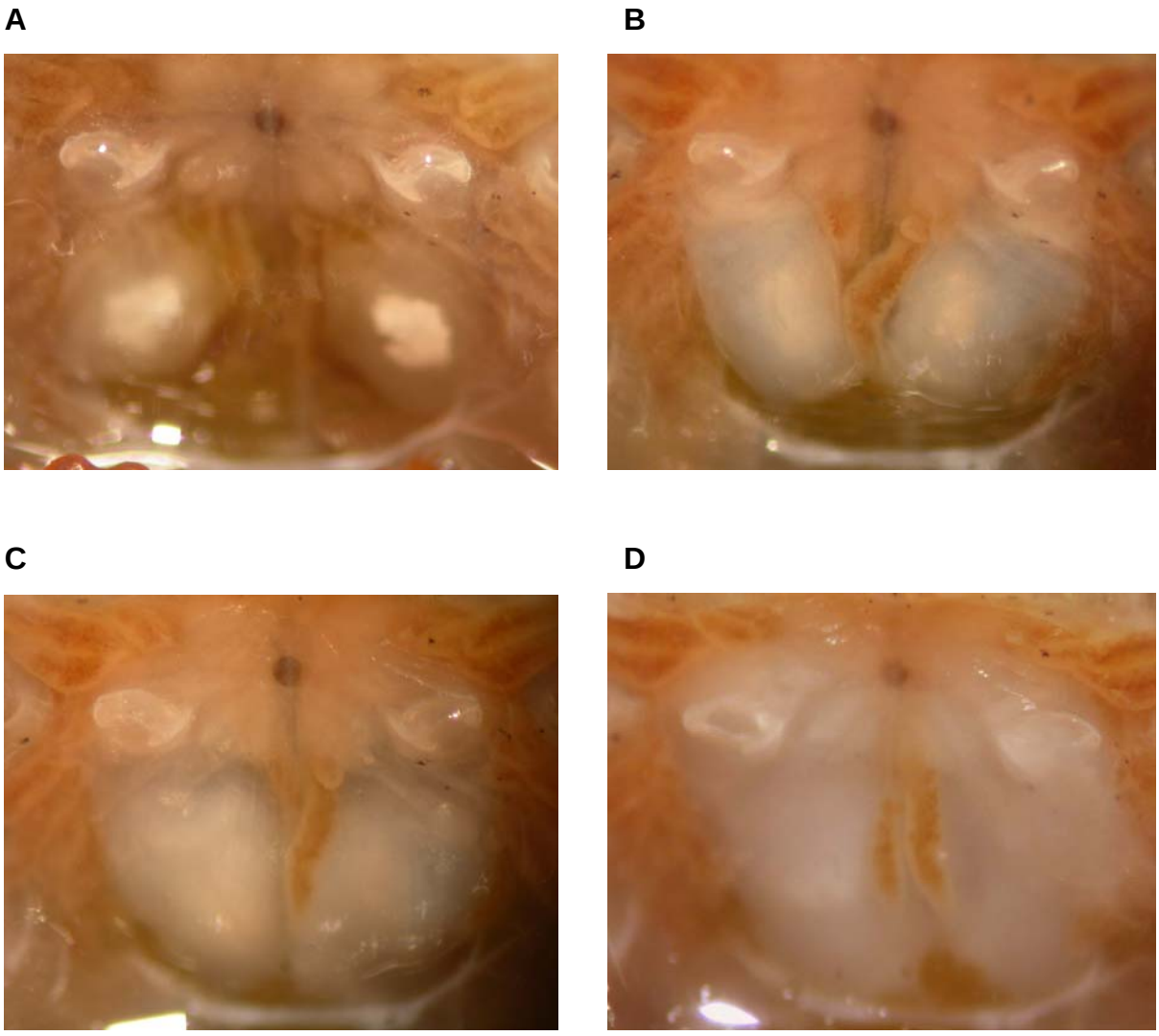


Plate 3.9 Digital photographs of the spermathecae of a single female after copulating with **(A)** no mates**, (B)** one mate, **(C)** two mates and **(D)** three mates.

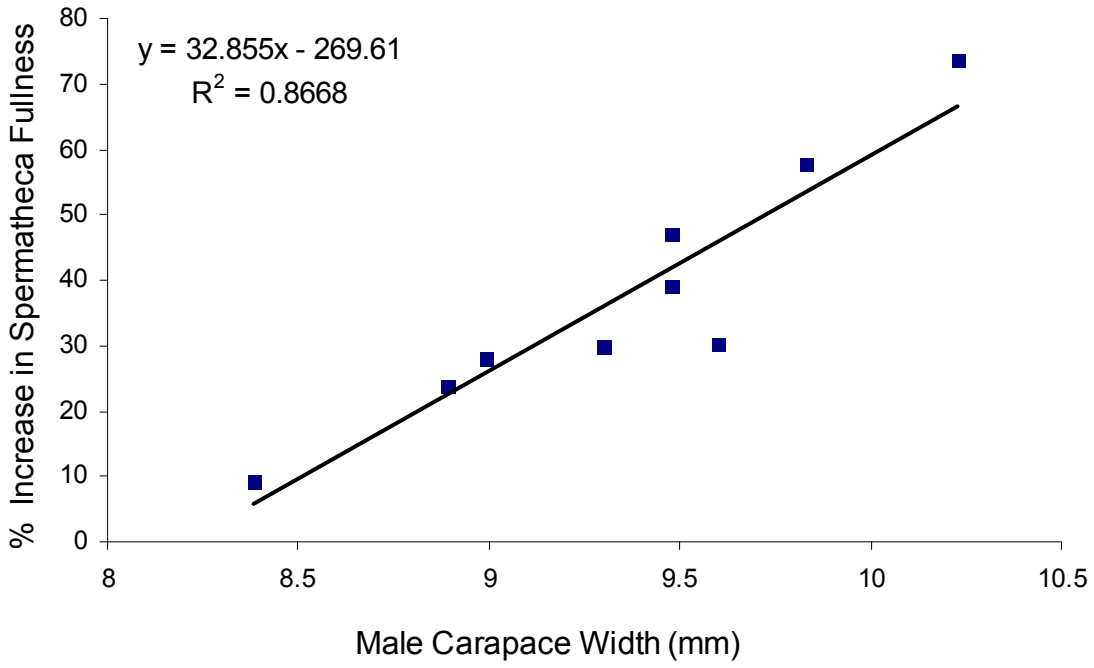


Figure 3.4 Relationship between male carapace width and the percentage increase in the fullness of the female's spermatheca (from visual estimate) after copulating.

After combining the results of the sperm supply and sperm use experiment, it was possible to produce an estimate of the proportion of a single ejaculate that females use to fertilize a single brood. As egg numbers were not recorded in an attempt to avoid high mortality, brood size could not be used as a factor influencing the proportion of sperm used in fertilization. Nevertheless, there was a difference of 15.03% between percentage increase and percentage decrease of mean visible spermathecae surface area, which suggests that females use approximately 15% of the ejaculate of one male to fertilize a single brood. Therefore, if a female has a spermatheca fullness visual estimate of 100%, she could theoretically fertilize at least 6 broods before sperm may become limiting.

Sperm mixing

Of the eggs carried by females who had only mated with healthy males, a mean of 0.6% were unfertilized, ranging from 0.12% to 0.99%. This indicated that the number of unfertilized eggs generally carried by females is negligible. Females in group A (those who had mated only with irradiated males) produced completely unfertilized broods. These eggs were often coloured a much duller orange when compared to fertilized eggs, and were lost within four days. This indicated that the radiation to which the males were exposed resulted in the successful and complete sterilization of the males.

80% of females in group B (those who had mated with an irradiated and then a healthy male) initially produced a normal sized, fertilized brood. The other 20% produced a smaller than usual, but fertilized brood. Six of the group B females produced a second brood during the experiment, all produced a normal sized brood initially, but within four days, the brood had reduced to approximately half its original size, but the eggs remaining were successfully fertilized. Two crabs were left with only about 20 fertilized eggs and one crab lost the entire brood. Three crabs produced a third brood during the experiment, but almost all eggs were lost save a few (50, 5 and 3 fertilized eggs in the three females).

Almost all the group C females (those who had mated with a healthy then an irradiated male) lost their entire first brood. Only three females had fertilized eggs in their first brood but only 13, 10 and 67 eggs were fertilized remained attached to the pleopods in these females. Four group C females produced a second brood, two completely lost their eggs within four days, and the remaining two carried 30 and 10 fertilized eggs to hatching. Two group C females produced a third brood, both of normal size. After four days, one was left with only one fertilized egg, and the other was left with approximately 50% of the original brood.

Incubation Time

The mean temperatures in the four rooms were 5.92°C ± 0.18°C, 10.31°C ± 0.21°C, 15.35°C ± 0.17°C and 20.28°C ± 0.096°C respectively. For convenience the temperatures will be referred to as 5, 10, 15 and 20°C.

Sample sizes for total incubation time only included those that completed an entire brood cycle, therefore sample sizes were less than the 20 originally stated. As no females completed an entire brood cycle in 5°C or 10°C, analyses were based on an accumulation of average durations for each individual brood stage and therefore show no variation.

Mortality was high at 20°C and successive brood stages appeared to decrease in size, an additional 5 females were added to compensate, but this was unsuccessful. Females entering the experiment with broods at stages 4 or 5 tended to lay another brood successfully, but females entering the experiment carrying broods at stages 1 or 2 tended to die before laying their next brood, or simply failed to lay a second brood. Mortality was also high at 5°C. One female at this temperature was observed to be carrying both stage 2 and stage 5 eggs simultaneously.

A comparison of total incubation time at different temperatures showed that incubation time was typical of related species, decreasing with increasing temperature (Figs. 3.5 and 3.6). The mean incubation duration in 5°C was 69.3 days, in 10°C: 43.8 days, in 15°C: 22.8 days ± 1.0 days and in 20°C: 14.7 days ± 1.2 days.

An ANOVA showed a significant difference in total incubation duration according to temperature ($F_{3, 14} = 226.361$, $p < 0.0001$). Each incubation time was significantly different from any other (Tukeys HSD $p < 0.01$ in all cases).

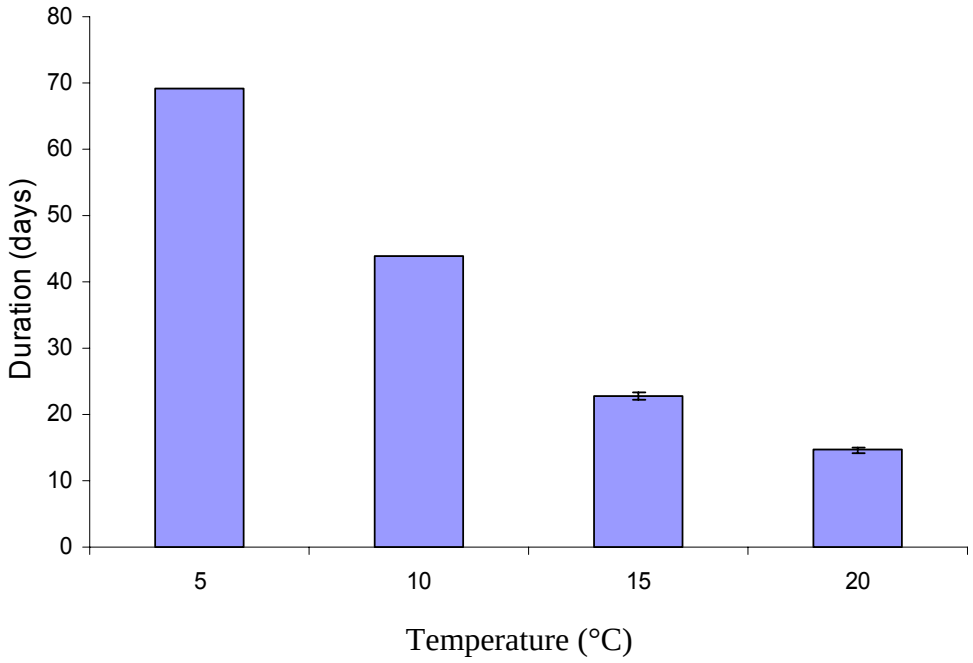


Figure 3.5 Mean incubation duration (days) of *Halicarcinus cookii* at different temperatures, ±1 S.E., n = 10 and 5 of 15°C and 20°C respectively. 10°C and 5°C have no error bars because they are not means, but estimates based on individual brood stages (see text).

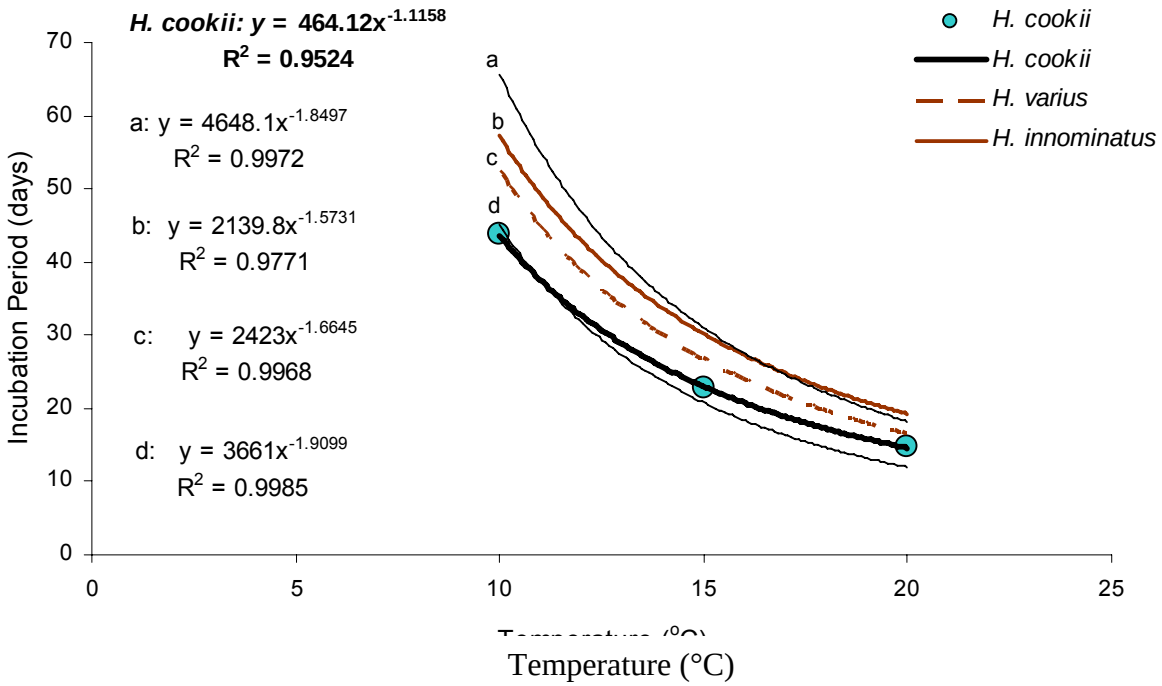


Figure 3.6 Mean incubation durations for *Halicarcinus cookii* at different temperatures compared with four other brachyurans: a) *Inachus dorsettensis*, d) *Galathea dispersa* (from Wear (1974), Table 4), b) *Halicarcinus innominatus*, (from Dunnington, 1999 Figure 3.3) and c) *H. varius* (from Hosie (2004) Figure 3.3).

The duration of each individual brood stage also decreased as temperature increased (Figure 3.7). Data for duration of each stage for each temperature were normalized with a square root transformation (Cochran p = 0.053) and a factorial ANOVA showed a significant difference in duration time of each brood stage according to temperature ($F_{3, 203}$= 368.65 (temperature), $F_{4, 203}$= 129.92, (brood stage) and $F_{12, 203}$= 6.84 (temperature × brood stage), $p < 0.001$ in all cases). For each temperature, stages 1 and 2 had the longest mean duration. Stage 3 had the shortest mean duration at all temperatures except at 5°C where stage 5 was shortest (Figures 3.7 and 3.8). At all temperatures, the duration of stages 3 and 5 remained most homogenous, although at 10°C the duration of stage 4 was also homogenous with stages 3 and 5 (Table 3.3). The mean percentage of total incubation time for each brood stage was calculated across all temperatures. Stage 1 had the highest mean duration of 34%, followed by stage 2 at 22%, stage 4 at 17% and stages 3 and 5 had the shortest mean duration of 13% and 14% respectively.

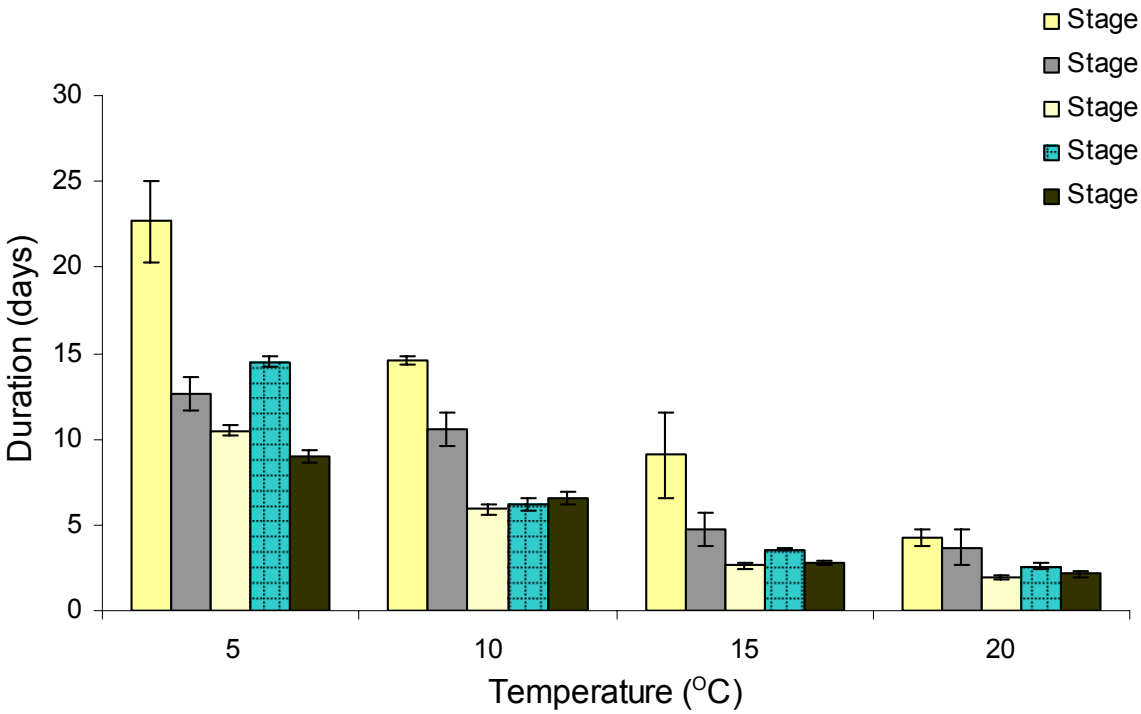


Figure 3.7 Mean incubation duration (days) of each individual brood stage (1-5) at different temperatures (°C), n = 4, 13, 18 and 16 for 5°C, 10°C, 15°C and 20°C respectively.

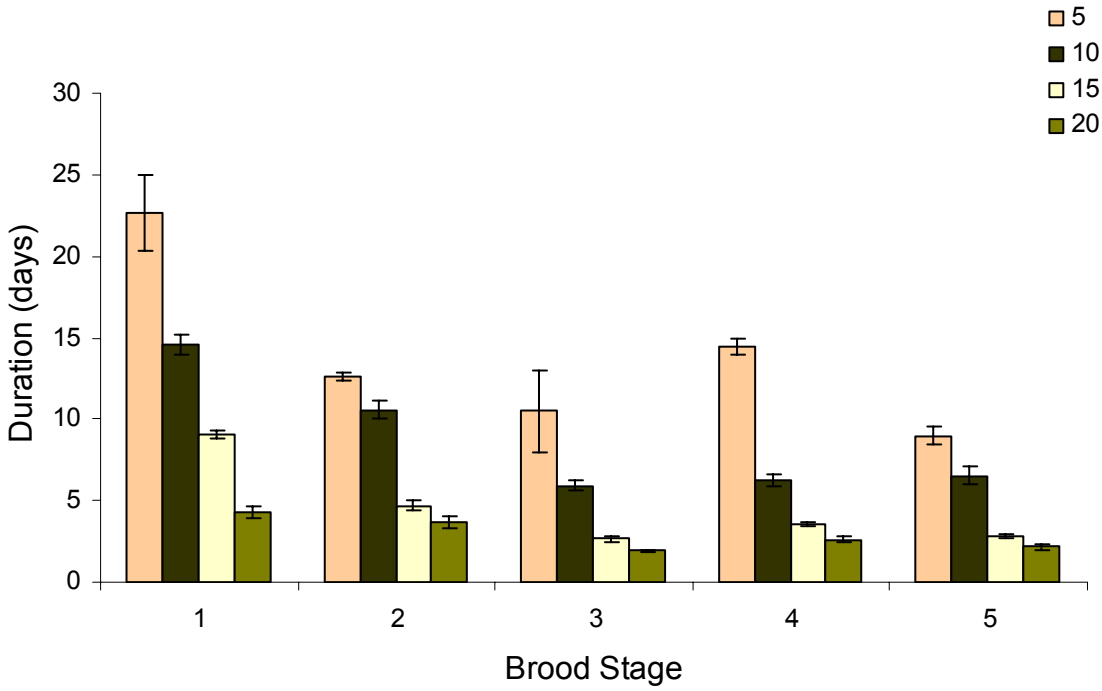


Figure 3.8 Mean duration (days) of each brood stage (1-5) ±1 S.E. at different temperatures (°C), n = 4, 13, 18 and 16 for 5°C, 10°C, 15°C and 20°C respectively.

Table 3.3 Mean number of days spent incubating eggs at each brood stage (1-5) in different temperatures (°C). Letters indicate mean durations not significantly different within temperatures (Tukeys HSD test p > 0.05). Roman numerals indicated durations not significantly different between brood stages at different temperatures (Tukeys HSD test p > 0.05).

	Temperature (°C)			
Brood Stage	**5**	**10**	**15**	**20**
Stage 1	22.7	14.57	9.05	4.29^{r}
Stage 2	12.6^{a}	10.57	$4.71^{e,l}$	$3.7^{f,g,l}$
Stage 3	$10.5^{a,b,c}$	5.91^{q}	2.65	1.91^{n}
Stage 4	$14.5^{b,c}$	6.21^{d}	3.58	$2.6^{g,h}$
Stage 5	9^{c}	6.5^{d}	$2.82^{e,II}$	$2.17^{h,II}$
Total	22.70	43.76	22.81	14.67

The mean inter-brood period also decreased with increasing temperature (Figure 3.9). The mean inter-brood periods were 2.33, 0.92, 0.28 and 0.25 days at 5, 10, 15 and 20°C respectively. An ANOVA showed a significant difference in inter-brood duration between temperatures ($F_{3, 34} = 9.45$, $p < 0.001$). Tukey's HSD test showed that inter-brood durations were significantly different between all temperatures ($p < 0.05$ in all cases) except for 15°C and 20°C ($p = 0.894$).

The mean sea water temperature in Kaikoura from December 2003 through to November 2005 was 12.9°C ranging from 8.77°C to 17.86°C (Figure 3.10). Over the summer months from November to April the mean temperature was 15.36°C, while over the winter months from May to October the mean temperature was 10.5°C.

Considering the mean temperatures experienced in the field and laboratory experiments, as well as the adult lifespan of *H. cookii* of approximately 6 months, (Chapter 2), an average female is likely to produce a maximum of approximately 8 broods in a life time. If an average female produces 1146 eggs per brood, she could be expected to produce 9168 eggs in a life time.

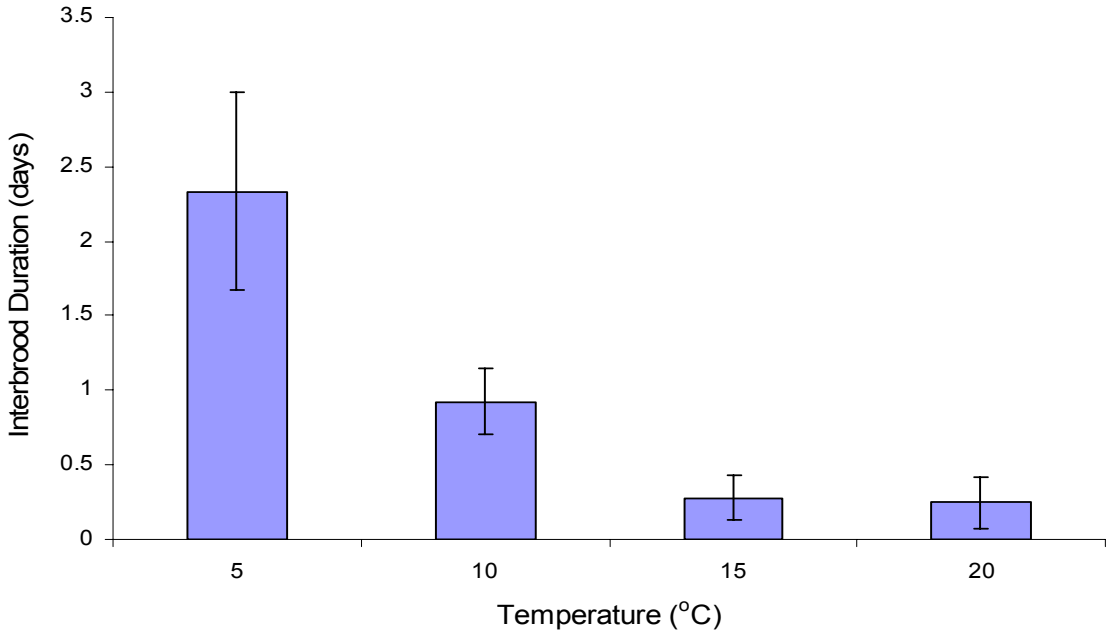


Figure 3.9 Mean duration (days) of the inter-brood period between larval release and oviposition of the following brood (stage 5 to stage 1) at different temperatures ±1 S.E.

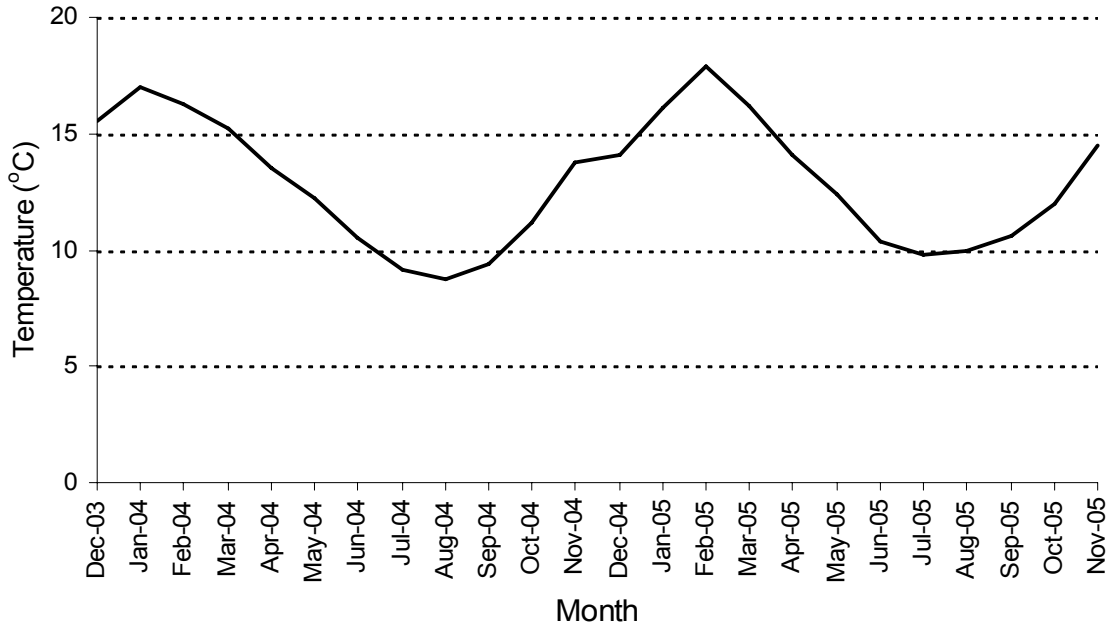


Figure 3.10 Mean sea water temperatures around the Kaikoura Peninsula from December 2003 to November 2005. Lines indicate the temperatures used in the incubation time experiments (5°C, 10°C, 15°C and 20°C). Sea temperature data funded by the Foundation of Research Science & Technology (FRST) and kindly provided by the National Institute of Water & Atmospheric Research Ltd (NIWA).

Pre-pubescent Maturity

Of the 30 males dissected, spermatozoa were definitely found in 23 (77%). These males ranged in CW from 6.33 to 11.42 mm. Spermatozoa were not seen in 4 males (13%) ranging in CW from 4.41 to 7.02 mm. The presence of spermatozoa was uncertain in remaining 3 males (10%), ranging in CW from 6.42 to 7.47 mm. The smallest male observed to mate had CW = 6.43 with a female with CW = 11.53. There is an apparent overlap in the presence of spermatozoa in males with CW between 6 and 8 mm, therefore, due to the lack of an obvious indication of male maturity such as propodus height, but males with CW > 7 mm were regarded as mature and those with CW < 7 mm as immature.

Females isolated during their penultimate instar moulted to maturity within 40 days of collection. 15 of the19 females produced a clutch of eggs within 3 days of moulting, while the remaining four produced a clutch within one week. Eight females produced a fertilized brood of eggs despite having no obvious sperm in the spermathecae and having been isolated from males before their maturity moult. The eggs produced by the remaining 11 females were assumed to be unfertilized as they were lost within 4 days.

3.4 Discussion

In Brachyurans, an individual's reproductive output relies on successful copulation, laying of eggs and incubation (Hartnoll, 1985). Hartnoll (1969) regards crabs as mature when they enter the inter-moult period and are first able to copulate successfully. For males, this involves the vas deferens containing spermatophores with large numbers of spermatozoa. For most species, male maturity is also indicated by an increase in chelae size relative to body size as seen in *Uca* spp. (Negreiros-Fransozo *et al*., 2003), *Heterozius rotundifrons* (Thompson, 1999) and *H. varius* (Hosie, 2004). Such a morphological difference in chelae size was not observed in *H. cookii* (Chapter 2). From the scanning electron microscopy photographs, there appeared to be little difference in the morphology of the mature male gonopod. For convenience, an estimate of 7 mm carapace width for male maturity had to be made for convenience during this study according to the presence of spermatophores observed during dissections. However, Hartnoll (1969) reported that spermatophore production in male crabs can occur before the assumed pubertal moult and Hosie (2004) observed morphologically immature males mating. From this study, male maturity in *H. cookii* was indistinct and can only be estimated.

In all brachyurans, female maturity is morphologically indicated by an increase in abdomen size relative to carapace width and development of a brood chamber over a single moult (Hartnoll, 1978). Some hymenosomatids experience a terminal, pubertal moult, so that once they reach sexual maturity they no longer moult (anecdysis), this is seen in the families Corystidae, Majidae and some Portunidae (Hartnoll, 1969).

According to Hartnoll (1965; 1969), females are theoretically not able to copulate as juveniles. In the Majidae, copulation prior to the pubertal moult is physically impossible as the gonopores are too small to permit mating. Similarly, the operculate gonopores of some grapsid species such as *Cyclograpsus lavauxi*, *Helice crassa*, *Hemigrapsus crenulatus* and *H. sexdentatus* restrict female receptivity to certain times when the operculum hinge line is decalcified (Brockerhoff and McLay, 2005a). However, some grapsids have no such physical restraints and the lack of precocial mating is attributed

to physiological factors. Hartnoll (1969) suggested that the hard shelled, precocial mating observed in females of the family Pinnotheridae several times before what would otherwise be considered the pubertal moult, were the only major exception to the general correlation of the pubertal moult and sexual maturity. In the Majidae, copulation prior to the pubertal moult is prevented by physical factors as the gonopores are too small to permit mating. Similarly, the gonopores of some grapsid species restrict female receptivity to certain times, such as *Cyclograpsus lavauxi*, *Helice crassa*, *Hemigrapsus crenulatus* and *H. sexdentatus* (Brockerhoff and McLay, 2005a). However, some grapsids have no such physical restraints and the lack of precocial mating is attributed to physiological factors. Hartnoll (1969) suggests that the hard shelled, precocial mating observed in females of the family Pinnotheridae prior to moulting several times before what would otherwise be considered the pubertal moult, are the only major exception to the general correlation of the pubertal moult and sexual maturity. Precocial mating and sperm retention was also observed in *Halicarcinus innominatus* (Dunnington, 1999) and *H. varius* (Hosie, 2004).

The SEM photographs showed a distinct difference in female gonopores and gonopods between penultimate instars and mature *H. cookii*. This suggests that there are physical restraints to precocial mating in this species. However, despite no obvious sperm in the spermathecae and isolation from males prior to, and following the pubertal moult, some female *H. cookii* produced fertilized broods. This suggests that precocial copulation can occur despite the potential physical restraints. Further investigations may clarify these seemingly contradictory observations. Precocial mating followed by the production of a fertilized brood suggests trans-moult sperm retention.

The development of the ovaries of sexually mature females is coordinated with egg production so that when the ovaries are at their most developed, they are ready to ovulate. Brachyurans are iteroparous, where an individual is able to produce many broods during its life time. There are two distinct patterns in brachyuran egg production. In species where females are only capable of copulating while soft shelled during an inter-moult period, reproduction occurs in well defined breeding seasons as seen in *Cancer magister* (Hankin *et al*., 1989) and *Chionoecetes opilio* (Sainte-Marie *et al*.,

2000). These species may produce a brood only once per year, ovary development and egg development are separate processes and females are barren for the majority of the year. Other species, where moulting and mating are not linked so that the females can mate and produce eggs in the hard shell condition, can produce multiple, successive broods in a single season and gonad development and brood development occurs concurrently. This synchronized gonad and brood development is seen in the Majidae such as *Chionoecetes opilio* (Sainte-Marie, 1993), *Mithrax sculptus*, *Macrocoeloma trispinosum, Pisa tetraodon* (Hartnoll, 1965), *Inachus phalangium* (Diesel, 1986) and *I. dorsettensis* (Bryant and Hartnoll, 1995). This pattern is also typical in the Hymenosomatidae such as *Hymenosoma orbiculare* (Broekhuysen, 1955), *Amarinus paralacustris* (Lucas, 1980) and *Halicarcinus lacustris* (Walker, 1969),

Halicarcinus cookii is typical of hymenosomatids in that female gonad development, as indicated by GSI levels, is synchronized with brood development. Successive broods were also generally laid within 24 hours of the previous one hatching in the range of temperatures experienced in the field. However, the hymenosomatids listed above have clearly defined breeding seasons in which the highest percentages of ovigerous females are found, whereas *H. cookii*, like *H. varius* (Hosie, 2004), has no such breeding season and ovigerous females are found throughout the year (Chapter 2). Some tropical majid species, such as *Microphrys bicornutus*, *Stenorhynchus seticornis*, *Mithrax sculptus* and *Macrocoeloma trispinosum* also show continuous brood production from the pubertal moult with high percentages of ovigerous females found each month (Hartnoll, 1965). High percentages of ovigerous *Chionoecetes* spp. and *Jacquinotia edwardsii* are found throughout the year, but due to the long developmental times (~12 months for *J. edwardsii*) females carry only one brood per year (McLay, 1988; Sainte-Marie *et al*., 2000). Hymenosomatids characteristically have short life spans likely to be around 12-18 months with 6 months as an adult (Hosie, 2004; Melrose, 1975). Therefore, this pattern of continuous egg production allows *H. cookii* to produce a maximum number of broods, estimated at about 8 broods, during their short life time.

In the temperate regime experienced by *H. cookii* in the field, successive brood production over a female's adult life exposes broods to a range of temperatures according to different seasonal changes in climate. Incubation times of decapod eggs are closely linked to the temperature of the water they are incubated in (Wear, 1974). In Kaikoura, the water temperature varies throughout the year due to seasonal changes in climate. *H. cookii* incubation times were typical of decapods in that incubation time decreased as temperature increased, probably due to an increase in the speed of metabolic processes with increased temperature (Leffler, 1972). However, as the temperature became too high (approximately 20°C) the adult crabs did not survive. When considering the observed incubation times and levels of fatality of *H. cookii* in the temperature rooms, it can be assumed that an optimal temperature for *H. cookii* to incubate eggs is close to, and perhaps slightly less than 15°C. Sea temperatures from December 2003 through to November 2005 in Kaikoura ranged from 8.77°C to 17.86°C, reaching over 15°C in only 8 of the 24 months recorded with an average of 15.36°C in the summer months and 10.5°C in the winter months. The mean sea water temperature during this time was 12.9°C, suggesting that *H. cookii* is well adapted to its environment regarding temperature as.

H. cookii seems to have a fast incubation time at any given temperature, particularly when compared to its close relatives *H. innominatus* and *H. varius*. At a mean temperature of 15°C *H. cookii* completed incubation in approximately 22.79 days, whereas *H. innominatus* completed incubation in approximately 30.22 days (Dunnington, 1999) and *H. varius* incubated in approximately 25.73 days (Hosie, 2004). At maximum mean temperatures of around 20°C *H. cookii* incubated their eggs in 14.67 days, faster than most other species studied such as *H. innominatus*: 22.3 days (mean temperature was 18.7°C) (Dunnington, 1999), *H. varius*: 16.88 days (Hosie, 2004), *H. ovatus:* 29 days, *Amarinus paralacustris*: 25.5 days (Lucas, 1980) and *A. laevis*: 29 days (Lucas and Hodgkin, 1970a). The percentage of total incubation time varied slightly from the estimates made in Chapter 2, but followed a similar trend. Stage 1 had the longest duration time, estimated at 44% and recorded as 34% of the total incubation time, followed by stage 2, estimated at 16% and recorded as 22%, stage 4 was estimated to have the shortest percent duration of 10%, but was recorded at 17%,

followed by stage 3, estimated at 12% and recorded at 13%. The estimate for the duration of stage 5 matched the observed result, suggesting that the duration of stage 5 comprises 14% of the total incubation time. This indicates that stages 3, 4 and 5 are the fewest in the population at anytime, and therefore the most limiting to males.

Among closely related species, greater eggs size increases the duration of incubation (Wear, 1974). Throughout the incubation period, egg size can change considerably; increasing as the eggs get closer to hatching (this study; Ali *et al*., 1995; Dunnington, 1999; Hosie, 2004). From the available literature, comparisons of incubation times while considering egg size of *H. cookii* can be made with *Elamenopsis kempi* (Ali *et al*., 1995), *H. innominatus* (Dunnington, 1999) and *H. varius* (Hosie, 2004). *H. cookii* had a relatively typical mean egg size of 0.025 µl for newly laid eggs when compared to hymenosomatids of similar size such as *E. kempi* (0.024 µl), *H. innominatus* (0.024 µl) and *H. varius* (0.017 µl). By the final stage of brood development, but before hatching, egg size had increased for all species, but *H. cookii* appears to have the smallest increase in egg volume: *H. cookii* (0.049 µl, 92% increase), *E. kempi* (0.058 µl, 141% increase), *H. innominatus* (0.066µl, 154% increase) and *H. varius* (0.034 µl, 101% increase).

The lower increase in egg volume for *H. cookii* may account for the faster incubation time at any given temperature when compared to *H. innominatus* and *H. varius*. If less energy is allocated to egg growth during development, more energy may be available to increase development time. However, egg size and percentage increase in volume of eggs for *H. innominatus* and *E. kempi* are similar, yet the rates of change in incubation time over a temperature regime are different. Dunnington (1999) estimated that at a maximum mean temperature of 18.7°*C*, *H. innominatus* would complete incubation in 22.3 days where *E. kempi* would incubate in 48 days. *E. kempi*, however occurs in sub tropical waters and is the only hymenosomatid species to incubate its eggs in a mean water temperature much greater than 20°C (ranging from 25-32°C) and at the minimum temperature incubation was completed in 23 days (Ali *et al*., 1995), suggesting that relative to their respective temperature regimes, *E. kempi* has a faster incubation time. This may simply be an indication that differences in incubation rates

between species are based on adaptations to their environments rather than egg size, with their optimal incubation times in synch with the temperature regime they experience in the field, and therefore incubation times cannot be compared between allopatric species using the same temperature regime.

The reproductive potential of a female is determined by the total number of offspring produced during her life, which is based on fecundity, number of mature instars, the number of broods produced per mature instar and the female's lifespan (Shields, 1991). Female body size is the principle factor influencing reproductive output in many brachyurans (Hines, 1982a; Hines, 1991). The large cancrid *Cancer anthonyi*, for example, produced an average of 2.208 million eggs per brood (Hines, 1992) while the smaller *C. oregonensis* produced and average 18.2 million eggs per brood (Hines, 1991). However, due to their small size, hymenosomatids have some of the lowest fecundity levels among the Brachyura (Lucas, 1980). In a single brood, *H. innominatus* produced between 512 and 2575 eggs (Dunnington, 1999) and *H. varius* produced 343-2350 eggs per brood (Hosie, 2004). Males would therefore be at an advantage to mate with larger females. The fecundity of *H. cookii* is typical of hymenosomatids and is highly variable, ranging from a brood size of 306 to 2438 eggs. 69% of the variation in egg numbers could be explained by female size. This suggests that female size is the primary determinant of brood size, but that other factors also influence the brood size of female *H. cookii*.

There are several possible factors influencing the number of eggs produced by female *H. cookii*. Variability in food resources may influence the number of eggs per brood. Large quantities of protein and lipid are essential for ovary development and the inability to acquire sufficient quantities may lower the fecundity of females (Wear, 1974). These substances are primarily obtained directly from food resources, but if these resources become limiting, as is possible in *H. cookii* with the successive brood production and the potential stress from captivity in the laboratory, lipid reserves from the hepatopancreas and protein from muscles may be redirected to the developing ovary, but may not adequately compensate, leading to a decrease in number of eggs produced (Wear, 1974).

Differences in fecundity levels may be a result of differences in the number of eggs produced by primiparous and multiparous females (Dunnington, 1999). Primiparous females are those producing their first brood after their pubertal moult, while multiparous females have already produced at least one brood. Lower fecundity in primiparous females is seen in *Chionoecetes opilio* (Sainte-Marie, 1993), *Inachus phalangium* (Diesel, 1988b), *I. dorsettensis* (Bryant and Hartnoll, 1995) and *Pyromaia tuberculata* (Furota, 1996). This difference in fecundity between primiparous and multiparous females has been suggested to be due to the partitioning of resources between competing processes of growth and reproduction or to the size limitation restricting the space available for ovary development in the primiparous female prior to the pubertal moult (Calow, 1978; Hines, 1982a; Hines, 1992). Dunnington (1999) suggested that it would be logical to assume that differences in fecundity between primiparous and multiparous females would be more pronounced in species where ovary development begins during the final juvenile instar, allowing brood production to occur immediately following the pubertal moult as seen in the hymenosomatids *A. paralacustris*, *A. lacustris* (Lucas, 1980), *H. innominatus* (Dunnington, 1999), *H. varius* (Hosie, 2004) and *H. cookii* (this study), rather than species that restrict ovary development until after the pubertal moult such as *I. phalangium* (Diesel, 1986) and *I. dorsettensis* (Bryant and Hartnoll, 1995). However, differences in fecundity levels occur in species showing both patterns. Therefore these differences are more likely to be a result of resource distribution than of physical size restraints. In *H. cookii*, females produced a brood of eggs within three days of the pubertal moult, suggesting that ovary development began prior to the pubertal moult. From this study, however, it is unknown whether there was a difference in fecundity between primiparous and multiparous female *H. cookii*, but if there was a difference, it may have been an influencing factor.

Egg or brood mortality may also cause the variation in egg numbers seen in *H. cookii*. Brood mortality in brachyurans can occur during any stage of development due to abrasion, lethal temperature and salinity levels, egg predators and parasites and maternal cannibalism (Somers, 1991). Egg mortality during development appeared to

be negligible for *H. cookii* as there was no significant difference in egg numbers between stages of development.

Egg loss can also occur during oviposition due to failure of the eggs to attach to pleopods or fertilization failure due to sperm limitation (Somers, 1991). Unfertilized eggs did not attach to the pleopods of *H. cookii* and were lost within 4 days. Females laid eggs regardless of the amount of available sperm, either stored or in a present male unlike in *Chionoecetes bairdi*, where females would not extrude eggs if there was insufficient sperm stored to fertilize a full brood (Paul, 1984). Loss of unfertilized eggs may account for the number of females found carrying no brood in the field as the mean inter-brood period in temperatures experienced by *H. cookii* was less than 24 hours, making them unlikely to be selected in the field for examination during the inter-brood period.

The short inter-brood period and potentially severe effect of a sperm shortage on the female's reproductive output may have been a selective force leading to the development of female sperm storage. Sperm storage is common in brachyurans and allows females to produce fertilized broods even if they do not encounter a male during the inter-brood period prior to oviposition (Hartnoll, 1985). From visual estimates, female *H. cookii* could fill their spermathecae to 100% after three or four copulations, but only used approximately 15% of the ejaculate of a single male to fertilize one brood. Although these estimates did not account for a change in spermatheca volume in the third dimension, and ejaculate size may depend on female sex ratio (Chapter 4) this is still a plausible estimate. Paul (1984) reported that wastage of sperm cells during fertilization is common in majids. Female *Chionoecetes opilio* used approximately 19% of the sperm cells in the spermathecae to fertilize a single brood and would not oviposit eggs with a sperm cell to oocyte ratio of less than ~7:1 (Sainte-Marie and Lovrich, 1994). With full spermathecae, a female *H. cookii* could potentially fertilize 6 broods without re-mating before sperm became limiting. If an average female produces around 8 broods in her life time, she may have to mate approximately 6 times to successfully fertilize all broods. Sperm storage in female *H. cookii* may therefore be an adaptation to compensate for low encounter rates in the field (Chapter 2).

There was a positive correlation in *H. cookii* between male size and the amount of ejaculate transferred to the female. In many crustaceans males tend to be larger than females, suggesting that there is selection for larger male size which leads to sexual dimorphism. The selective force behind larger male size is likely to be primarily male-male competition, of which the transfer of more sperm is likely to be included. By transferring more sperm, a male is indirectly competing with rivals by increasing his potential to fertilize more eggs. Larger male *Gammarus pulex* were more successful at mating than smaller males (Bollache and Cezilly, 2004). Likewise, large male fiddler crabs (*Uca paradussumieri*) had greater success in mating and longer copulation times than smaller males, suggesting more sperm was transferred, than their smaller counterparts (Jaroensutasinee and Jaroensutasinee, 2003). After mating with larger male spiny lobsters (*Jasus edwardsii*), females produced significantly larger broods than after mating with smaller males (MacDiarmid and Butler, 1999).

The increase in visual estimates of spermatheca fullness over the year lagged slightly behind that of the number of males found. This may be explained by the short life span and peak in population size of *H. cookii*. There was an obvious increase in the number of both mature females and males found in the beginning of summer (Chapter 2), suggesting that there were large numbers of females that had just completed their pubertal moult. As the number of mature females increased greatly compared to males in the summer months, the high mature female to male ratio may have led to a low encounter rate and, therefore, a low copulation rate in the field. This suggests that it took the females a few months to encounter enough males to fill their spermathecae in the summer. By the winter females had encountered enough males so that their spermathecae were close to full. Furthermore, male numbers decreased as winter approached, suggesting that the sperm supply may have become limited so that females were unable to mate frequently enough to fill their spermathecae compared to summer.

Another possible explanation is that due to the short life span of the female, the mature female to male ratio at the end of summer and during winter was much lower, leading

to a higher encounter rate for those surviving during this time. The more severe weather and wave action during the cooler months may also increase the encounter rate by forcing the animals to aggregate in the available shelters. A reduction in brood production and therefore sperm use during the cooler period when the decreased temperature increased incubation time may also be a factor influencing differences in spermathecae fullness throughout the year. In a study of *Jasus edwardsii*, MacDiarmid and Butler (1999) suggested that females could avoid sperm limitation by multiple mating prior to oviposition, mating as early in the reproductive season as possible or mating with large, preferably unmated males. This may therefore also be the case for *H. cookii*. However, from this study, only speculations can be made and further investigations are required if a more conclusive explanation is to be offered.

The spermathecae of female *H. cookii* were obviously ventral type in that the oviduct and vaginal opening were placed ventrally and close together. Eggs pass through the ventral section of the spermathecae and encounter the sperm of the last male to mate (Diesel, 1991). This suggests that *H. cookii* shows last male sperm precedence. Dunnington (1999) reported clear evidence of sperm storage in *H. innominatus* through histological analysis, but evidence of sperm layering within the spermathecae was inconclusive. Separate layers of ejaculates can form within the spermatheca in species where males inject spermatophores surrounded by seminal plasma that harden after transfer into the female's spermatheca, such as the spider crab, *Inachus phalangium* (Diesel, 1991) and the snow crab, *Chionoecetes opilio* (Sainte-Marie and Sainte-Marie, 1999). In such species, the sperm that fertilizes the eggs will come almost exclusively from one male. However, in *C. opilio*, there is evidence for multiple paternity of broods when several ejaculates co-occur close to the oviduct opening (Sainte-Marie *et al*., 2000). By using irradiated males, Koga *et al*. (1993) found that in the sand bubbler crab, *Scopimera globosa*, the last male to mate could fertilize 94.1% of the eggs in a brood. Although dense sperm packets could be seen in *H. innominatus*, the layers were not obviously separated, and sperm mixing may have occurred (Dunnington, 1999).

H. cookii appears to show little sperm mixing, at least for the first brood. This is plausible if a female only uses 15% of an ejaculate as discussed above. For the second

and third broods, however, females who had mated with a healthy then a sterile male seemed to show a small degree of sperm mixing in that females that laid a normal to small sized brood lost all but a few eggs which remained attached to the pleopods and developed, indicating they were fertilized. Likewise, females who had mated with a sterile then healthy male produced a normal sized, fertilized brood initially, but in their second and third broods, eggs were lost leaving only some fertilized eggs attached to the pleopods. This indicates that there is some, albeit small degree of sperm mixing in *H. cookii*. Sperm mixing is relatively common among arthropods. In the haplogyne spider *Pycnosorus globosus,* there is a degree of sperm mixing which leads to the possibility of several males fertilizing one brood of eggs (Uhl, 1998). The weevil *Diapreres abbreviatus* shows last male sperm precedence and sperm mixing, so that the last male to mate with a female fertilizes about 75% of the eggs (Harari *et al*., 2002).

The apparent absence of a mixed paternity brood initially may be due to the amount of sperm transferred filling enough space in the spermathecae so that the eggs do not come in contact with sperm from previous mates. This may be further evidence for selection for larger males that can transfer more sperm, thus diluting or displacing rival sperm inside the spermathecae. Time required for mixing may also be a factor resulting in mixed paternity of the second and third broods. Females generally laid their first brood after mating within 24 hours, while their second brood was laid an entire incubation period after the female's copulation. Therefore during the incubation of the first brood, the sperm in the spermathecae may have had time to mix leading to the observed mixed paternity in the second brood. From this study, the occurrence of sperm mixing in *H. cookii* can be established, but the degree of sperm mixing and proportion of paternity is still unclear.

The reproductive characteristics of *H. cookii* are relatively typical of the Hymenosomatidae. Penultimate instar ovary development allows females to produce eggs immediately following the pubertal moult. Despite a small brood size compared to other brachyurans, fecundity is increased with successive rapid brood development, possible with synchronized ovary and brood development maximizing the number of

broods produced per life span. Water temperature directly affects the duration of brood incubation with incubation time decreasing in increasing temperatures. Temperature therefore influences the number of broods a female can produce per life span. Characteristic of brachyurans, sperm storage in *H. cookii* allows females to produce multiple fertilized broods without re-mating, thus ensuring a high reproductive output despite low male encounter rates. Sperm mixing inside the spermathecae appears to occur to some degree indicating that sperm competitin occurs in this species.

Chapter 4

Reproductive Behaviour

4.1 Introduction

Brachyuran reproductive strategies are highly variable as they are designed to maximize individual reproductive fitness and compensate for the limitations of the biology and population structures of each species (Diesel, 1991). Over the last few decades, the reproductive biology of brachyurans has been the focus of many studies, both for large, commercially important species such as *Callinectes* spp. (Jivoff, 1997a; Jivoff, 1997b), *Chionoecetes* spp. (Donaldson and Adams, 1989; Elner and Beninger, 1995; Moriyasu and Comeau, 1996; Paul and Paul, 1996; Watson, 1970) and *Cancer* spp. (Edwards, 1966; Elner *et al*., 1985; Kittredge *et al*., 1971), as well as species with no commercial value, but provide additional examples of the variable reproduction, mate choice and sexual selection related behaviours exhibited in the Brachyura. These include the spider crabs *Hyas coarctatus, Inachus dorsettensis* (Bryant and Hartnoll, 1995) and *Libinia emarginata* (DeGoursey and Auster, 1992), and Hymenosomatids (Chuang and Ng, 1994; Lucas, 1980) such as *Halicarcinus innominatus* (Dunnington, 1999), *H. varius* (Hosie, 2004) and *Rhynchoplax coralicola* (Gao *et al*., 1994).

The variability in brachyuran reproductive strategies primarily relates to the differences in moulting patterns of each species (Hartnoll, 1969; Hazlett, 1975). Hartnoll (1969) pointed out that in all brachyurans, the male gonopods must be able to successfully penetrate the female gonopores. Therefore, all males must have a hard exoskeleton in order to successfully copulate. Mature females of many brachyuran species, however, have a direct link between moulting and reproductive receptivity. Typically, females are

physically able to mate only when their exoskeleton is soft, immediately after moulting, as is common in Cancridae and Portunidae (Berrill and Arsenault, 1982; Edwards, 1966; Edwards, 1979; Elner *et al*., 1985; Hartnoll, 1969). In other species, females are able to mate during the intermoult period (when the integument is hard), but only when the hinged operculum covering the gonopore is decalcified and therefore mobile (Brockerhoff, 2002; Hartnoll, 1969), such as the grapsid crabs *Gaetice depressus* (Fukui, 1993), *Cyclograpsus lavauxi*, *Helice crassa*, *Hemigrapsus crenulatus* and *H. sexdentatus* (Brockerhoff and McLay, 2005). In species where copulation is possible at such restricted times of only a few days when the exoskeleton is soft or the operculum decalcified, the timing of copulation is critical to the individual's reproductive success (Hartnoll, 1969).

Still other species show female receptivity following a terminal, pubertal moult in either the soft or hard shell condition. After this moult to maturity, females become reproductively active and growth ceases. Although majids reproduce seasonally, they can mate without moulting. Female majids are able to copulate and produce their first brood immediately following the pubertal moult, even before their exoskeleton hardens (primiparous), they can then produce the following broods in a hard-shell condition (multiparous) (Diesel, 1986; Diesel, 1988b; Elner *et al*., 1985). In the Hymenosomatidae, females show continuous brood production and hard shell mating throughout their adult life (Lucas, 1980). Although this strategy allows much less restriction on mating opportunities, the cessation of growth restricts brood size (see Chapter 3) and the ability to replace damaged limbs (Hartnoll, 1969).

Female mate choice has rarely been studied in species that have no obvious male pre-copulatory courtship. Male fiddler crabs, *Uca paradussumieri* attract females with displays such as waving chelipeds or by building burrows. In this species there is evidence for female mate choice, with females preferring larger males (Jaroensutasinee and Jaroensutasinee, 2003). Lucas (1980) suggested that the conspicuous colouring of some male Hymenosomatids may be evidence for visual displays. Melrose (1975) observed agonistic displays between males involving the spreading of chelipeds and gripping of each other in *Halicarcinus varius*, *H. whitei* and

H. innominatus, but did not suggest that this related to female choice. Mate choice in most brachyuran species appears to be evident only in males.

Mate guarding is common in Brachyura. Mate guarding often involves the male caging the female beneath him, grasping her carapace or legs with his chelae or simply remaining in contact prior to, or immediately following copulation (Diesel, 1988b; Hartnoll, 1969; Lucas, 1980). Hartnoll (1969) identified two basic patterns of mate guarding behaviour in brachyuran species according to the degree of association between moulting and mating (this also depends on the structure of the spermathecae as described in Chapter 3). In species where the female can only mate in the soft-shell condition, such as in the families Cancridae and Portunidae, a male may guard the female until she moults, then copulate with her and resume guarding until the female's integument has hardened. Hartnoll (1969) suggests that such guarding protects the male's reproductive investment into the female in two ways: 1. by protecting the vulnerable, soft-shelled female from predation and cannibalism, and 2. by preventing rival males copulating with the female. Alternatively, in species where multiparous females can mate in a hard-shell condition, there is often little pre-copulatory guarding, but the male may guard the female for a time following copulation. As females of these species are not especially vulnerable to predation, post-copulatory mate-guarding is likely to result from male-male competition.

Guarding behaviour appears to be relatively plastic according to the population structure. To maximize their reproductive fitness, males will attempt to fertilize as many eggs as possible, while females, if given a choice, should prefer to fertilize their eggs with sperm from the best quality male, or to ensure a constant sperm supply and therefore mate with multiple males (Diesel, 1991; Dunnington, 1999; Emlen and Oring, 1977; Jormalainen and Merilaita, 1995; Parker, 1974; Wada *et al*., 1999). The most efficient strategy or amount of time invested into mate guarding may change if the operational sex ratio is skewed to either extreme. Males have to contend with higher competition for mates in a male biased operational sex ratio, whereas in a female biased operational sex ratio, the encounter rate for potential mates is high and male-male competition is lower, allowing them to invest less energy in any individual female

(Christy, 1987; Jormalainen and Merilaita, 1995). Males guarded females for longer under a male biased sex ratio than those under a female biased sex ratio in *Heterozius rotundifrons* (Thompson and McLay, 2005) and the hermit crab, *Pagurus middendorffii* (Wada *et al*., 1999). Jivoff and Hines (1998) found that sex ratio had a similar effect on male blue crabs, *Callinectes sapidus*.

In order to maximize their reproductive fitness, males must discriminate between females that are most economical in terms of requiring the least time investment into guarding to ensure their paternity of the next brood and allow more time to search for new mates and females that are not worth the effort. Most investigations into the cue used by males to recognise a reproductively receptive female have focused on species with soft-shell mating and the use of female sex pheromones as a secondary purpose for, or linked to the moulting hormone (Bamber and Naylor, 1997; Christofferson, 1978; Gleeson, 1991; Kamio *et al*., 2000; Kamio *et al*., 2002; Ryan, 1966; Seifert, 1982).

Studies of species with a terminal moult and hard-shell mating have shown that males preferentially mate with females about to spawn and closest to laying a new brood. This male mate choice is seen in *Inachus phalangium* (Diesel, 1988b), *Halicarcinus innominatus* (Dunnington, 1999) and *H. varius* (Hosie, 2004). In studies of these species, the presence and nature of a cue was difficult to identify. Furthermore, the source of the cue was also in question, whether the males recognise a cue from the females body (such as from developed ovaries) or from the brood of eggs about to hatch. Dunnington (1999) attempted to identify whether the cue came from the eggs or the female by observing the behaviour of males in the presence of two females after their eggs had been removed. One female had been carrying late stage eggs and the other had carried recently laid eggs. He found that male behaviour was similar to what he had observed when the eggs had not been removed, and concluded that the cue came from the female herself, but whether this cue was olfactory or tactile remained inconclusive.

There has been some recent work on the reproductive behaviour of Hymenosomatidae including Dunnington (1999), Hosie (2003), Chuang and Ng (1994) and Gao *et al*

(1994), but studies are very limited compared to other brachyuran families. In a survey throughout Australia, Lucas (1980) reported that in the family Hymenosomatidae, females were capable of both mating in the soft-shell condition immediately after moulting (primiparous) and subsequently mating when the integument was hard (multiparous). *Hymenosoma orbiculare* females were only able to mate in the soft-shell condition and moulting continues throughout their life (Broekhuysen, 1955; Lucas, 1980). In these species, the male guarded the female prior to moulting and until the integument had hardened. Alternatively, female *Halicarcinus varius* (Hosie, 2004) and *H. innominatus* (Dunnington, 1999) experience a terminal, pubertal moult and mate in the hard-shell condition. These species showed little pre-copulatory guarding, but post-copulatory mate-guarding was obvious (Dunnington, 1999; Hosie, 2004; Lucas, 1980).

As with other *Halicarcinus* species, *H. cookii* experiences a terminal, pubertal moult and females are able to copulate in the hard-shell condition (Melrose, 1975). However, Melrose (1975) reported that there was no evidence of courtship in this species and that a mating pair will separate soon after copulation. My study will be the first in-depth investigation of the reproductive biology and behaviour of *H. cookii*. Various aspects that may potentially influence mating behaviour will be investigated, including the possibility of female choice, the effect of the stage of development of the brood carried by the female on male mating behaviour, the effect of altered sex ratio on male behaviour and an investigation into the cue that males use to recognise ‘attractive’ females.

4.2 Methods:

Study Subject Husbandry

For all mating behaviour experiments, *H. cookii* were collected from the field in different areas to those designated as monthly population sample sites so that the population survey would not be influenced by the removal of individuals for experiments. Crabs were brought to the lab, separated by sex and placed in punctured 2 L ice cream containers in a flow-through tank supplied with purified seawater kept at ambient temperature, until they were required for mating trials. Every 3-4 days the crabs were fed a mixture of crushed gastropod snails (*Zeacumantus* spp., *Austrolittorina* spp. and *Melagraphia aethiops*) and mussels (*Perna canaliculus* and *Mytilus galloprovincialis*).

Mating Behaviour

For each trial, females were selected according to the stage of development of the brood they carried in their brood chamber. Broods were categorised into six stages; stage 0-5 (see Chapter 3 for descriptions of brood stages) and juvenile females were described as stage 'J'.

A female carrying a brood of known stage and a male were selected and placed together in a 2 L ice cream container half filled with fresh seawater. Each pair was monitored for 60 minutes for copulation to occur. To aid these observations, each mating trial was recorded using a Panasonic™ WV-BP312 infrared video camera and a Panasonic™ AG-1070 time-lapse video recorder, which allowed up to 24 hours of constant recording and avoided any effects of changes in the lighting regime and human interference. A copulation was identified when the male had turned himself onto his back and positioned his sternum inside the brood chamber of the female whom he clasped from underneath with his legs. Despite a lack of evidence that sperm transfer invariably occurs every time a pair are in this copulation position, it was assumed to be the case. If no copulation occurred within 60 min, the trial was terminated and recorded as 'no mating', but if copulation did occur, its duration was monitored, as well as the duration of any post-copulatory physical contact termed 'mate guarding'. Mate guarding was recorded when the male caged the female, with his sternum on the females

carapace, or simply when the male's legs or chelipeds remained in contact with the female. The pair was monitored until the male lost physical contact with the female and all mating behaviour between the pair was considered to have ended. All observations were recorded in decimal minutes. 20 trials for each brood stage were conducted producing a total of 140 trials. New crabs were used for each successive trial to maintain a degree of independence in the results. The frequencies and durations of mate guarding for each brood stage was compared using one way ANOVA and a χ^2 test for goodness of fit. Throughout the behaviour trials, observations of male *H. cookii* were made to determine the sequence of behaviours involved in mating and the various courses of action a male may take when encountering a female.

During population surveys (Chapter 2) instances of mate guarding found in the field were recorded as an anecdotal confirmation that laboratory results could be extrapolated and applied to what naturally occurs in the field.

Operational Sex Ratio

When it was established that males preferentially mated with and guarded females carrying late stage eggs, the experiment was modified to determine whether or not the presence of other males influenced the males mating behaviour. Mating trials, similar to those described above were conducted with a late stage female and two males of similar size. Timing began when one male clutched the female and ended when he lost physical contact with her. Observations of male-male interactions were also recorded. The duration of copulation and mate guarding in the presence of a second male was then compared to that of the single male trials.

The same experiment was then run again with two stage 5 females of similar size and one male to determine any difference in behaviour when there is a late stage female biased sex ratio. As above, timing began when the male grasped a female and ended when physical contact was lost. The female first grasped by the male was monitored to determine if her brood hatched before that of the other female.

Female choice

To determine whether or not the female had any choice or influence over which male she mated with, two males of obvious size difference (small males: approximately 8 mm CW, large males: over 12 mm CW) were selected, measured and then tethered to opposite corners of a 2 L ice cream container filled to ¾ with seawater. The crabs were tethered by attaching one end of a length of thread to their carapace with quick-dry superglue and tying the other end to the container. The males were given 10 min to acclimatize before the female was placed in the middle of the container inside a small transparent container with holes punctured in the sides so that the female experienced the same water as the males. After a further 10 min acclimatization period, the container was removed allowing the female to freely roam the container. The size class of the first male to clutch the female was recorded. 20 trials using new crabs each time were conducted and results were compared.

Nature of Attractant

The nature of the cue provided by the female and detected by the male to indicate female attractiveness was investigated. Pieces of chamois cloth were wrapped around a small stone and tied shut. These were then placed in a container with late stage females and 50 ml fresh seawater for each female and left for 24 h. After 24 h a male was placed in a 2 L ice cream container half filled with fresh seawater and then presented with one chamois covered stone conditioned with female water and one that had not been in water. The chamois chosen by the male was recorded. A chamois was considered to have been chosen if the male grasped onto it with his legs, not when he simply backed up to it. The results were recorded as a binomial ‘yes’ or ‘no’ choice rather than duration of time spent at each chamois due to the possibility of the crab remaining in contact with the chamois simply because it was an object on which to cling. 22 trials were conducted, and a new male was used for each trial.

The experiment was modified to control for the possible preference of a chamois that had been in water to one that had been sitting in air prior to the trial, or for a preference for a chamois that had been in the presence of conspecifics, either male or female, than for one that had not. The same trial as described above was conducted again, but

with one chamois stone conditioned in water with stage 5 females and one that had been conditioned in water with stage 1 females for 24 h. The chamois chosen by the male was recorded. 44 trials were conducted and a new male was used for each trial

Again the experiment was modified to control for the possible attraction of the male to females regardless of brood stage or ovary development. 44 trials as described above were carried out, using a new male for each trial, with one chamois stone conditioned with late stage females and one that had spent 24 h in water with other males.

Source of Attraction

An experiment was designed to determine the source of the male attractant to these females; whether the male responded to the female or the embryos. Females carrying stage 5 broods were subjected to the videoed guarding trial as described above, and then the embryos were removed with care to avoid any damage to the pleopods. The trial was then conducted again with a different male, and the female without embryos.

The embryos were placed in a 30 × 20 mm length of dialysis tubing, sealed at both ends and presented to a male who was then observed using a time-lapse video recorder as described above. If the male clasped the dialysis tubing with his legs, he was considered to have interest in it and the duration of this contact was recorded.

To determine whether or not a male could distinguish between a female with developed ovaries and one with undeveloped ovaries, two females, one with a stage 5 brood, and one with a stage 1 brood were placed in a container, after the brood had been removed. 20 trials were run, each with a different male introduced into the container and his choice of which female to mate with was recorded. The broods of stage 4 and 5 females were carefully removed and the females presented to males individually and observed using the time lapse video technique described above. The duration of copulation and mate guarding were recorded and compared between brood stages.

4.3 Results:

Mating Behaviour

Mating behaviour in *Halicarcinus cookii* was observed to be relatively uniform, varying only slightly within an identifiable set of behaviours (Fig. 4.1). Males tended to make the decision of whether the female was worth pursuing or not early in the encounter, but a female could be rejected at any time before or after mating. There were two courses of decision making observed by the male after taking interest in a female. Either a male would notice that a female was attractive almost immediately, advancing toward her and clutching onto her with quick, jerky movements as soon as physical contact was made. Alternatively, the male would have one or two legs in contact with the female for some time (often a few minutes) before 'realising' the female was worth his interest and then clutch onto her body, legs or chelipeds. This grasping position, termed 'guarding' involved the male on top of the female's carapace with his legs wrapped around the female's body and sometimes one or both of the male's chelae clutching her legs or chelipeds. The male usually faced the same direction as the female, and would remain in the grasping position regardless of whether the female moved around the container.

Once grasping the female, the male moved to adopt the classic copulation position (Plate 4.1). This involved crawling to the underside of the female, turning on his back and inserting his abdomen between the female's sternum and abdomen, inside the brood chamber. It was assumed at this point that the male was transferring sperm through his gonopods into the gonopores of the female. Once the male had completed copulating (usually after 30-50 minutes), he would assume a post-copulatory mate guarding position by returning to the grasping position, standing over the female and 'caging' her with his legs or dismounting the female but maintaining contact with one or more of his walking legs (Plate 4.2). The occurrence of post-copulatory mate guarding and length of time this position was held depended on the stage of development of the brood that the female carried (see below). The male would then leave the female and show no further interest in her.

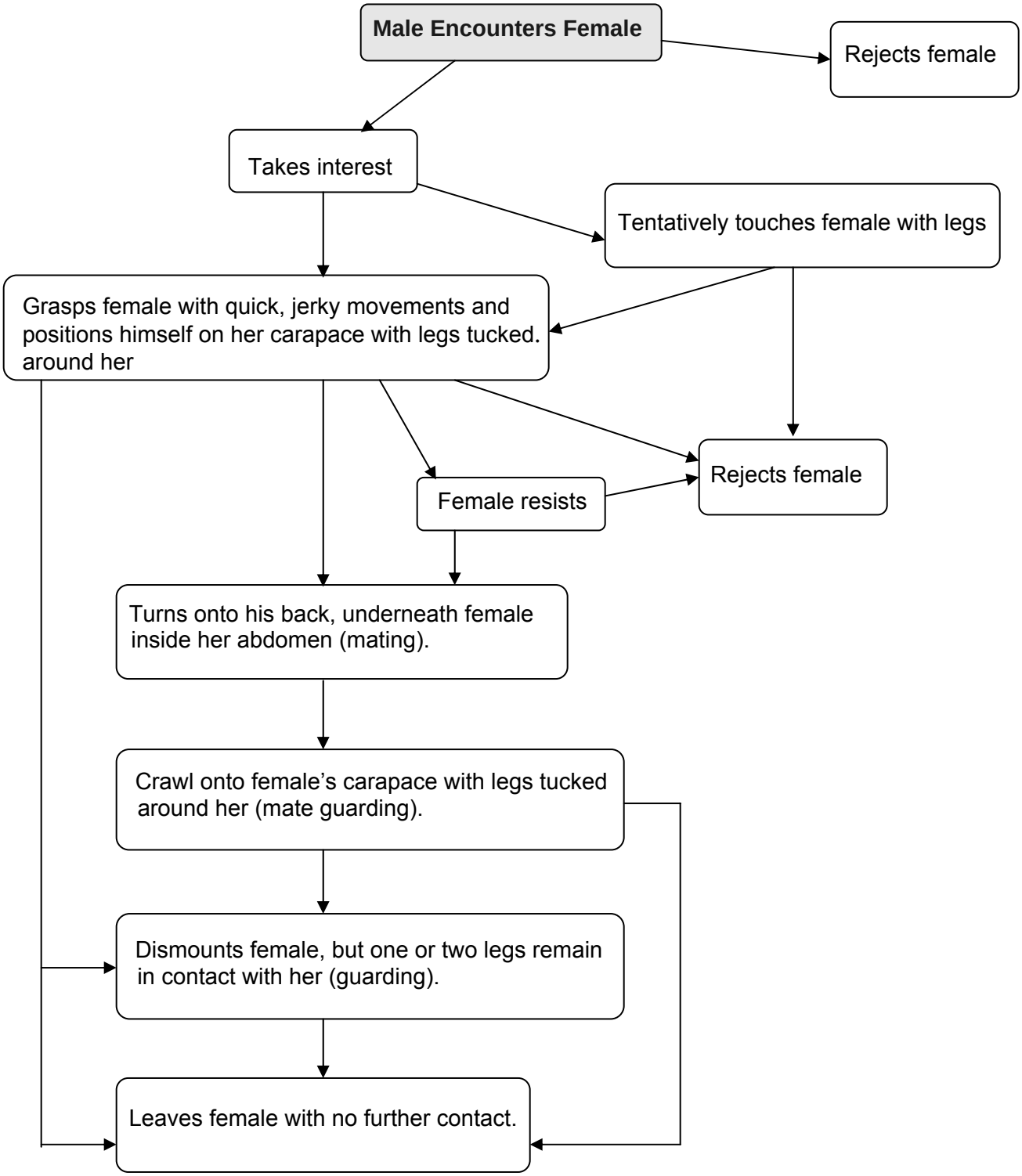


Figure 4.1 Flow chart outlining the mating behaviour of *Halicarcinus cookii* as observed in the laboratory.

A

B

Plate 4.1 Pair of *Halicarcinus cookii* male and female in copulatory position with the male turned on his back and grasping the ventral side of the female from **(A)** Dorsal view and **(B)** Side view (note (C) where the male is inside the open female abdomen).

A

B

Plate 4.2 Male and female *H. cookii* in typical post-copulatory guarding position with the male on the dorsal side of the female, grasping her with his legs from **(A)** dorsal view, and **(B)** anterior view.

Occasionally a female appeared to resist the advances of a male by avoiding physical contact or moving around and struggling when grasped by the male, sometimes even using her chelipeds in defence. In these cases the male would either give up on his advances at any stage of mating or, if he could overpower her, grasp the female and mate with her regardless.

Mate Choice

Copulations were observed to occur with females carrying any brood stage (stages 0-5). Only one copulation with a juvenile female was observed, but in several cases males appeared to attempt to mate, but failed. Males appeared to mate most frequently with mature females carrying broods close to hatching (stages 4 and 5) followed by females carrying no brood (stage 0) (Fig. 4.2). With all brood stages pooled, 59 copulations were observed out of a total of 140 encounters. A χ^2 comparison of observed and expected copulation frequencies indicated that the frequency of mating was not random in regard to brood stage, but varied accordingly ($\chi^2 = 32$, df = 6 $p < 0.001$). Mean copulation time was 37.5 minutes. There was no obvious difference in copulation time according to female brood stage (Fig. 4.3). An ANOVA indicated that the durations were homogeneous ($F_{5,36} = 1.43$, $p > 0.05$).

Over the population survey (Chapter 2), at least 8 instances of guarding were observed in the field, all of which involved females carrying stage 5 broods. Post-copulatory mate guarding was observed in the laboratory with females at all brood stages, but was most common with females carrying late stage broods (Fig. 4.4). A χ^2 comparison between observed and expected frequencies of mate guarding showed that the occurrence of mate guarding was not random, but was strongly dependent on female brood stage ($\chi^2 = 34.847$, df = 6, $p < 0.001$).

Although post-copulatory mate guarding was observed with females carrying broods at all stages, the duration of mate guarding varied accordingly. There was an obvious preference for much longer mate guarding of stage 5 females than earlier stage

females (Fig. 4.5). Variances could not be homogenized with data transformations so the data was log transformed to produce the closest to homogenous variances possible and the threshold for significance was increased to $p < 0.01$. An ANOVA revealed a strongly significant difference in mate guarding durations according to brood stage ($p < 0.001$). Post-hoc analysis (Tukey's HSD test) showed that mate guarding duration of stage 5 females was significantly different from all other stages ($p < 0.001$ in all cases) while all other stages were not different ($p > 0.05$ in all cases).

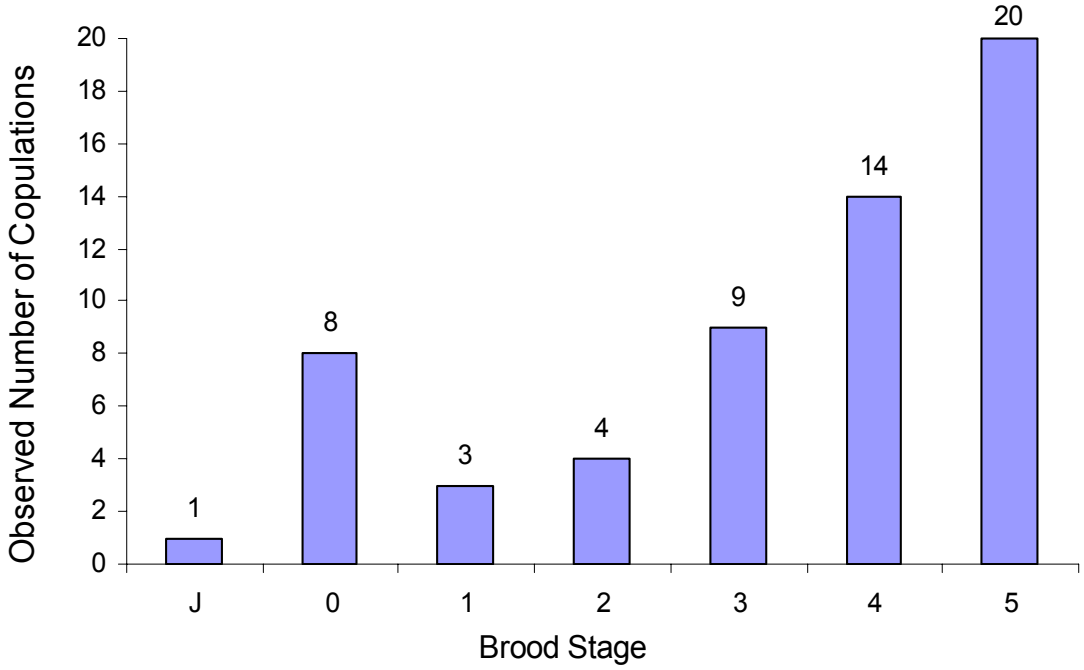


Figure 4.2 Observed frequencies of *H. cookii* encounters resulting in copulation between males and females carrying different brood stages (0-5) and juvenile females (J). Data labels indicate observed frequencies. A total of 59 copulations were observed out of 140 trials.

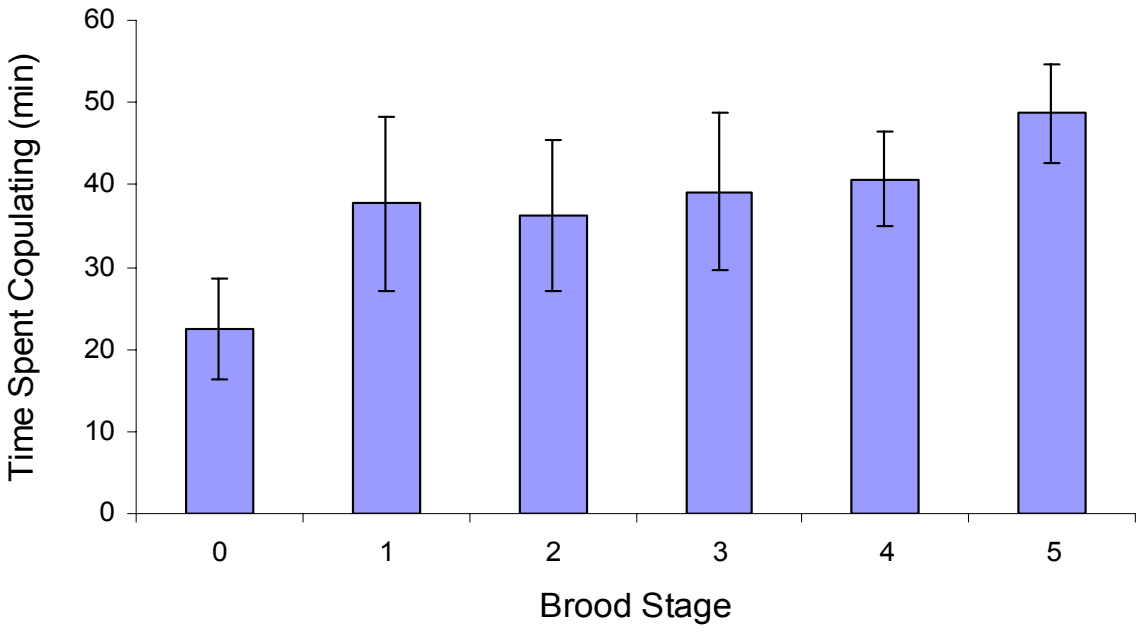


Figure 4.3 Mean duration of copulation (±1 S.E) with females carrying different brood stages (0-5).

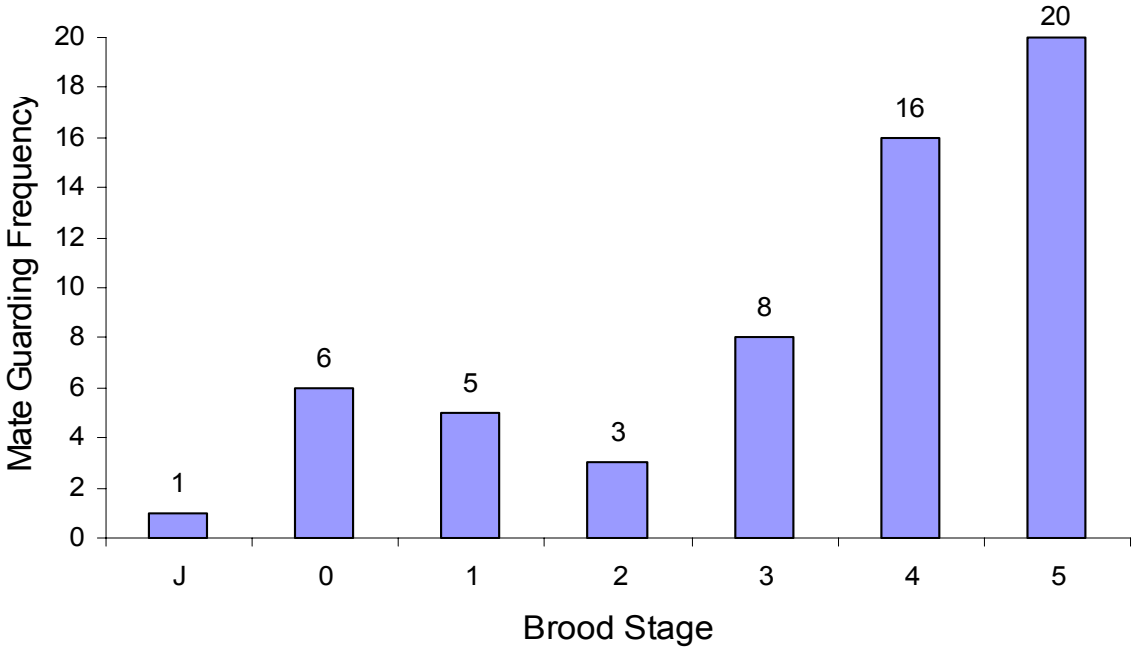


Figure 4.4 Observed frequencies of post-copulatory mate guarding with females carrying different brood stages (0-5) out of 20 trials for each brood stage. Data labels indicate actual number of observed mate guarding incidents.

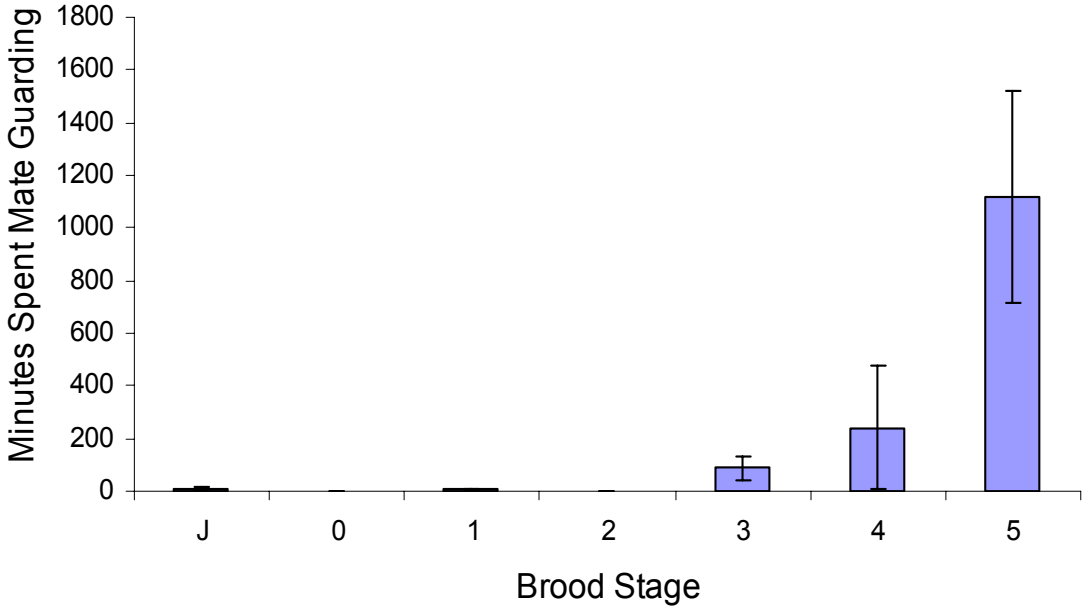


Figure 4.5 Mean duration (minutes) of observed post-copulatory mate guarding (±1 S.E) by males with females of different brood stages (0-5).

Sex Ratio

Agonistic behaviour was observed between the two males when both were present with a single female. These displays occurred when males faced each other and stood on the dactyli of their walking legs, lifting the front of the carapace upwards, spread their chelipeds and occasionally clutched onto their opponents appendages. Eventually one male would retreat or both appeared to lose interest. When checking the experiment, the female was often found alone while the two males were regularly found clutching onto each others carapace with their legs and chelipeds but not obvious damage was caused (plate 4.3).

Plate 4.3 Typical display of agonistic behaviour between two males, often occurring in the presence of a desirable female. Note the stance and splayed chelipeds. The third crab present (right) is a female.

When a single male was in the presence of two stage 5 females, the male would generally mate with the first female he came across. The male copulated with both females in about 40% of the trials, and would more often lose interest in both females after mating/guarding one, or clutch onto the second female but not copulate, sometimes alternating between the females. Occasionally one of the two females' broods would hatch, and she would lay a new brood, after which she was no longer attractive to the male. The male did not mate preferentially with the female whose brood would hatch first. Sometimes the male appeared to be undecided about which female to mate with and would attempt to clutch onto both of them, after which one would eventually be released and copulation with the other would begin.

Copulation duration with the 1:2 ratio (ratios are presented as males per female) was much shorter than with the other two ratios (Fig. 4.6). Males copulated for a mean of only 6.5 minutes in the presence of two females while copulation lasted a mean of 33 minutes and 31 minutes in the 1:1 and 2:1 situations respectively. Data on the duration of copulation could not be normalized (Cochran $p = 0.04$) so the threshold for significance was raised to $p = 0.01$. There was a significant difference in copulation duration between the three sex ratios ($F_{2,30}= 23.60$, $p < 0.001$). Post-hoc analysis with a Tukey's HSD test revealed that copulation duration between the 1:1 and 2:1 ratios were homologous ($p > 0.05$) but there was a significant difference in copulation duration in the 1:2 ratio ($p < 0.001$).

Post-copulatory guarding duration increased as the male to female ratio increased (Fig. 4.7). In the two female situation, mate guarding lasted a mean of 2.5 hours compared to the 1:1 situation where guarding lasted a mean of 20 hours and in the 2:1 ratio, 29.6 hours. There was a significant difference in guarding durations when all three sex ratio situations were considered ($F_{2,30}= 30.8$, $p < 0.001$). When only comparing the single male and two male situations, the difference in guarding duration was significant (Tukey's HSD: $p < 0.001$). However, a Tukey's HSD test for all three situations showed that the 1:2 ratio was significantly different from the other two situations ($p < 0.001$), but the sole and double male situations were not significantly different ($p > 0.05$). The most variation was seen in the 1:1 situation.

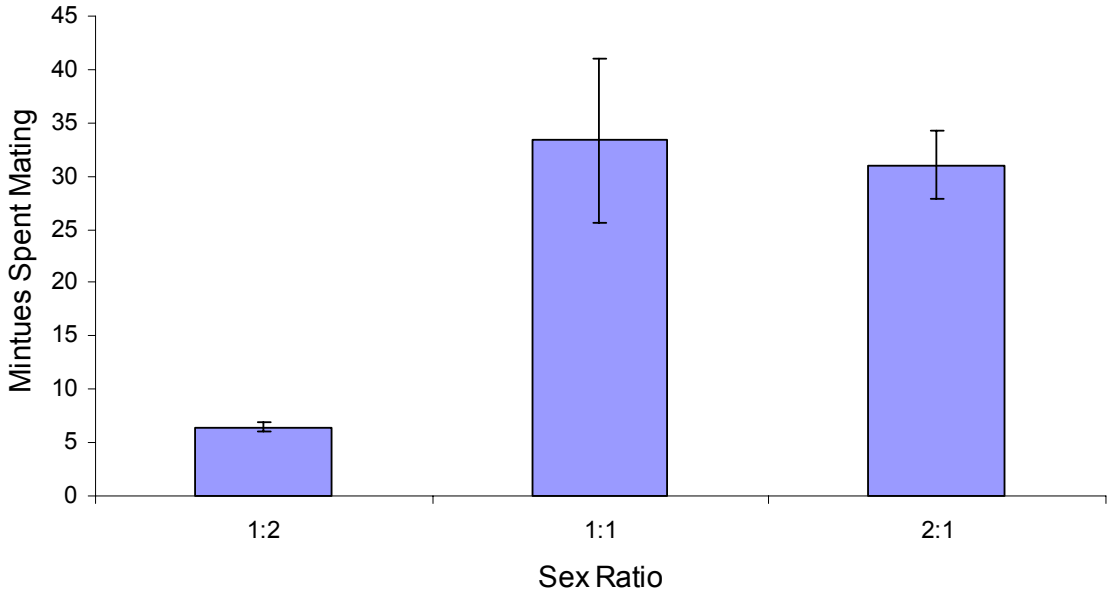


Figure 4.6 Comparison of mean copulation durations (minutes) (±1 S.E.) between different sex ratio situations. Ratios are male per female.

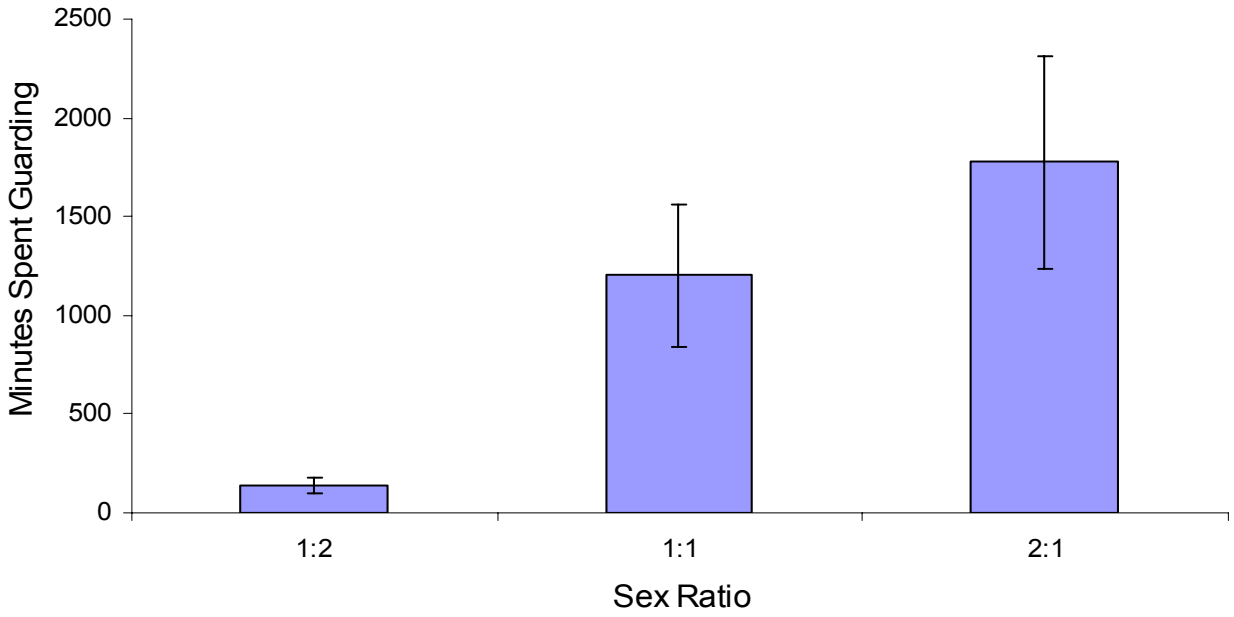


Figure 4.7 Comparison of mean guarding durations (minutes) (±1 S.E.) between different sex ratio situations. Ratios are male per female.

Female Choice

When 15 females were faced with the choice of a male larger than themselves and a male smaller than themselves, there was no significant difference in choice made between the males (Cochran's Q = 2, df = 1, $p > 0.05$).

Source of Attraction

Males preferentially chose to mate with females carrying late stage broods over early stage broods even after the embryos were removed (Cochran Q =7.12, df =1, $p < 0.05$). A comparison of copulation and guarding duration was made between females at stage 4 and 5 before and after their broods were removed. A factorial ANOVA showed that males copulated with females regardless of whether or not they carried embryos, and there was no significant difference in the occurrence of copulation depending on whether the female carried a brood or not or between the two brood stages ($F_{1.39}$= 3.35, $p = 0.075$ (brood stage); 1.92, $p > 0.05$ (brood presence) and 2.27, $p > 0.05$ (brood stage × brood presence)). A factorial ANOVA showed that the duration of copulation was not significantly different between when the female carried a brood and when it were removed, but there was a significant difference between brood stages ($F_{1,39}$= 19.01, $p < 0.001$ (brood stage); 0.34, $p < 0.05$ (brood presence) and 0.6, $p > 0.05$ (brood stage × brood presence)).

Males generally guarded females of both brood stages regardless of whether or not the females carried a brood. A factorial ANOVA showed no significant difference in the occurrence of mate guarding between females with and without a brood ($F_{1,32}$= 0.62 (brood stage); 0.03 (brood presence) and 0.03 (brood stage × brood presence), $p > 0.05$ in all cases).

Variances in mate guarding duration could not be homogenized so data were logged to produce the closest to homogeneous variances possible and the threshold of significance increased to $p < 0.01$. A factorial ANOVA showed a significant difference in mate guarding duration according to brood stage, but no significant difference according to egg presence ($F_{1,39}$= 9.92, $p = 0.003$ (brood stage), 6.9, $p > 0.01$ (brood presence) and 6.86, $p > 0.01$ (brood stage × brood presence)). However, Tukey's HSD

test showed that there was a significant difference in guarding time between stage 5 females with broods, and each of the other three conditions ($p < 0.01$ in all cases). A one way ANOVA was then conducted to compare stage 5 females with and without broods and showed a significant difference in guarding duration ($F_{1,19} = 18.81$, $p < 0.001$).

No males showed interest in the embryos enclosed in dialysis tubing other than one male who succeeded in ripping open the plastic and eating them.

Males appeared to prefer chamois covered stones that had been in water with late stage females compared to those that had been left in air for 24 hours. A Cochran Q test showed a significant difference in male choice between these to chamois stones ($Q = 16$, $df = 1$, $p < 0.001$). However, when given the choice of a chamois stone that had been in water with late stage females or in plain seawater, there was no significant difference in male decision ($Q = 0.03$, $df = 1$, $p > 0.05$). There was also no significant difference in male choice made between chamois stones that had been in water with late stage and early stage females ($Q = 1.4848$, $df = 1$, $p > 0.05$) or between chamois stones that had been in water with late stage females and with males ($Q = 1.2$, $df = 1$, $p > 0.05$).

4.4 Discussion

Reproductive behaviour is designed to compensate for the limitations of population structures and physical or physiological restraints (Diesel, 1991). Due to the wide range in biology and population dynamics within the Brachyura, reproductive behaviours are highly variable among brachyuran families, including males using visual or auditory signals to attract females as seen in fiddler crabs, *Uca* spp. (Hazlett, 1975; Koga *et al*., 1993; Salmon, 1967), or prolonged attendance of females, either before or after copulation, or both such as *Chionoecetes opilio* (Berrill and Arsenault, 1982), *Heterozius rotundifrons* (Thompson, 1999) and *Cancer pagurus* (Edwards, 1966). *Halicarcinus cookii* males showed little evidence for visual displays for attracting females (Melrose, 1975, this study), but males commonly guarded females after copulation.

Male chelipeds in *H. cookii* grow significantly larger than female chelipeds (Chapter 2) and larger males tend to transfer more sperm than smaller males (Chapter 3). This suggests that larger males are more successful than smaller males, indicating that there is some selective force behind the development of this sexual dimorphism. Sexual dimorphism results from sexual selection and can develop through two main mechanisms, mate choice and competition for mates (Andersson, 1994; Andersson and Iwasa, 1996). Male fiddler crabs commonly attempt to influence female choice with sexual displays where males wave their major chelae in defined patterns to attract females, such as *Uca stylifera, U. stenodactyla* and *U. beebi* (Hazlett, 1975). In *U. paradussumieri*, the development of large body size in males was attributed to both a female preference for larger males and male-male competition (Jaroensutasinee and Jaroensutasinee, 2003). There was little evidence for female mate choice in *H. cookii* on male size. Compared to males, females appeared almost completely passive during reproductive encounters. Although at times females seemed to struggle and resist the advances or grip of males during either copulation or guarding, all other reproductive and agonistic behaviour was exhibited by males. Therefore, the sexual dimorphism observed in *H. cookii* can be attributed predominantly to male-male competition, and studies on the reproductive behaviour of *H. cookii* should focus primarily on males.

The primary influence on the type of male reproductive behaviour exhibited by a species is female receptivity, which occurs in two patterns. In most brachyurans, females must have a soft integument in order to mate successfully, resulting in females only mating in the intramoult period, this is common in the Cancridae (Berrill and Arsenault, 1982; Edwards, 1966; Edwards, 1979; Elner *et al*., 1985; Hartnoll, 1969) and is seen in portunids such as *Portunus sanguinolentus* (Christofferson, 1978; Ryan, 1966) and *Carcinus maenas* (Bamber and Naylor, 1997; Hardege *et al*., 2002; Seifert, 1982). In these species, males may guard a female prior to her moult to both ensure her safety during such a vulnerable period and guarantee him an opportunity to mate once the female has moulted. In these species, female receptivity is short and lasts approximately 3 days depending on the species (Hartnoll, 1965; Hartnoll, 1969). This short period of receptivity results in few available mates for searching males, so males invest long periods of time into pre-copulatory mate guarding. Male *Heterozius rotundifrons* may guard a female 5 days before her moult (Thompson, 1999; Thompson and McLay, 2005). Pre-copulatory mate guarding in *Carcinus maenas* lasts an average of 6 days and up to 16 days prior to the females moult (Berrill and Arsenault, 1982).

In other species, females are able to mate in the hard-shell condition so that female receptivity is not linked to moulting. In some species, females are able to mate during the inter-moult while the majority of the integument has hardened, but the hinge attached to the operculum covering the gonopore is soft enough to allow the entrance of the male gonopod (Brockerhoff, 2002; Hartnoll, 1969). This is seen in the grapsid crabs *Gaetice depressus* (Fukui, 1993), *Cyclograpsus lavauxi*, *Helice crassa*, *Hemigrapsus crenulatus* and *H. sexdentatus* (Brockerhoff and McLay, 2005a). In these species males will only guard females with soft opercula. In species where females experience a terminal/pubertal moult, females are able to mate and produce eggs continuously with a hard integument, so they are no more vulnerable during this time than any other (Diesel, 1986; Diesel, 1988b; Elner *et al*., 1985). This strategy is seen in some majid species such as *Inachus phalangium* (Diesel, 1986; Diesel, 1988a) and *Chionoecetes* spp (Donaldson and Adams, 1989; Paul, 1984; Stevens *et al*., 1994) and in some hymenosomatids such as *Halicarcinus innominatus* (Dunnington, 1999) and *H.*

varius (Hosie, 2004). In these species, pre-copulatory guarding by males was rare, or short lived. Instead males guarded females after copulation for varying lengths of time to ensure his paternity of the next brood by preventing rival males mating with the female. Likewise, *Halicarcinus cookii* is among those species where moulting and mating are not linked and females are able to mate at any time after their pubertal moult.

In any species, males attempt to maximize reproductive fitness by mating with as many females as possible. However, in some species males may lose paternity of the brood if another male mates with the female. Through guarding, a male can prevent other males mating with the female, but guarding females limits the time available for a male to search for more mates. Furthermore, guarding a female for a lengthy time increases energetic requirements and restricts feeding opportunities for the male (Jormalainen, 1998; Parker, 1974). Therefore, there is a trade off between the benefits of mating with many females and potentially producing more offspring, with the risk of low female encounter rates and losing paternity of a brood to a rival male, or ensuring paternity through mate guarding while reducing mating opportunities and increasing survival costs. Consequently, males must discriminate between those females worthy of time invested into guarding and those where the cost of guarding is too high.

In species with soft-shell mating, males simply need to identify females that are close to moulting. Several studies have suggested that males identify these females as ‘attractive’ by the release of a moulting hormone that can potentially double as a sex pheromone such as crustecdysone, a chemical released from a pore at the base of the females antennae with the urine (Bamber and Naylor, 1997; Christofferson, 1978). This is not a species specific hormone and can therefore incite pre-mating behaviour in males of various species. This was shown in the grapsid *Pachygrapsus crassipes*, and the cancrids *Cancer antennarius* and *C. anthonyi* (Kittredge *et al*., 1971). When released from female *Pachygrapsus crassipes*, this pheromone caused male *Cancer antennarius* and *C. magister* to attempt copulation with pre-moult female *C. producta* (Christofferson, 1978). Crustecdysone has also been shown to elicit copulation attempts by males with other males in *Heterozius rotundifrons* (Thompson, 1999),

stones in *Carcinus maenas* (Hardege *et al*., 2002), and in *Telmessus cheiragonus*, males successfully inseminated sponges soaked in water containing females (Kamio *et al*., 2000; Kamio *et al*., 2002). Methyl farnesoate, synthesized by the mandibular organs, has also been suggested as a reproductive hormone, as it controls development in larval and juvenile stages and reproduction in adults (Laufer and Ahl, 1995; Laufer *et al*., 1992; Laufer *et al*., 2002; Laufer *et al*., 1987). In *H. cookii*, females can mate without moulting, therefore a moulting hormone cannot double as a sex pheromone in this species. Therefore the use of methyl farnesoate in *H. cookii* reproduction is plausible, but currently unknown.

Without the possible use of a moulting hormone due to the hard-shell mating in *H. cookii*, males must identify 'attractive females' (those worth the time investment of guarding) through other means. The only factors that appeared to change in the reproductive life of a mature female *H. cookii* were the stage of brood development and ovary development. There was a trend indicating that males preferred to mate with females carrying more developed broods. Only 15% of encounters resulted in copulations with stage 1 females compared to 100% of stage 5 females. The duration of copulation however, showed no change according to female brood stage. This suggests that males choose mates according to brood stage, but may be unable to alter the amount of sperm transferred if copulation occurs. This pattern was also seen in *H. varius* (Hosie, 2004), *H. innominatus* (Dunnington, 1999), *Inachus dorsettensis* (Jones and Hartnoll, 1997) and the hermit crab *Pagurus filholi* (Goshima *et al*., 1998).

Similarly, male *H. cookii* showed a preference for guarding stage 5 females over all other females. 100% of stage 5 females were guarded by their mate compared to 20% of stage 1 females and 15% of stage 2 females. Although post-copulatory mate guarding occurred with females carrying broods of all stages, the duration of guarding was significantly longer for stage 5 females than any other female with a mean of 19 hours. During the guarding of a stage 5 female, the female's existing brood sometimes hatched and a new brood was laid, after which the female was released by the male who then showed no further interest in her. Due to last male sperm precedence in *H. cookii* (Chapter 3) females carrying broods closest to hatching are also closest to

fertilizing a new brood using the sperm of their last mate. Therefore, it is in the interest of males to invest time into guarding stage 5 females rather than any other female. By choosing females whose broods are closest to hatching, males attempt to prevent wasting sperm and time on females who are likely to re-mate before a new brood is fertilized and reduce sperm competition (Jivoff, 1997a; Parker, 1970). Many other species with hard-shell mating show a similar trend including *H. varius* (Hosie, 2004), *H. innominatus* (Dunnington, 1999) and *Inachus dorsettensis* (Jones and Hartnoll, 1997). However, when presented with two stage 5 females, the choice of which female to mate with appeared to be random in *H. cookii*, suggesting that males were unable to accurately determine which of the two females would spawn first and that they have incomplete information about the details of brood development.

The obvious preference that *H. cookii* males show for females carrying broods about to hatch indicates that males are able to identify these females despite the lack of a moulting hormone acting as a sex pheromone. Little evidence of the nature of the cue used by males to identify 'attractive' females could be established. Although the cue is assumed to be waterborne, the chamois cloth experiment failed to support this, suggesting the possibility of a tactile signal. If such a waterborne chemical cue exists in *H. cookii*, it may either be too weak, or degrade too quickly after emission to be adequately absorbed by the chamois cloth. This scenario is similar to that of *H. innominatus*, where Dunnington (1999) tried to identify the nature of the cue released by 'attractive' females, but was unable to come to a reliable conclusion. Similarly, Jones and Hartnoll (1997) reported that male *I. dorsettensis* recognised females carrying late stage broods through a waterborne pheromone, but was unable to elaborate further.

The source of this chemical cue is equally uncertain. Diesel (1986) suggested that the ripe eggs in the ovaries may be a source of attractant in *Inachus phalangium*, but Jones and Hartnoll (1997) found than in *I. dorsettensis*, females who have recently hatched their eggs were also attractive. In *H. cookii* the presence of a brood had little influence on the attractiveness of females. Males preferred to mate with and guard stage 5 females over stage 1 females even when their broods had been removed. The

difference in guarding duration between stage 5 females with and without a brood may be due to the males sensing the absence of a brood and behaving as if the brood had hatched.

If the brood stage indicates female attractiveness, but the presence of a brood is not the source of attractant in *H. cookii*, there must be another factor on the same time scale as brood development generating female attractiveness. The development of the brood in *H. cookii* coincides with that of the gonads (Chapter 3), suggesting that the ovaries are a possible source of the attractant. Males may detect a cue that indicates that the ovaries are ripe and close to extruding a new brood of eggs. This has been suggested as a source of pheromone in other species such as *Libinia emarginata* (Hinsch, 1968) and the lobster *Homarus americanus* (McCleese *et al*., 1977). Although *Corystes cassivelaunus* experiences a terminal/pubertal moult, their ovaries were still immature immediately after ecdysis, at which time females did not mate. Only when the ovaries had matured were the females attractive to males (Hartnoll, 1968). However, Jones (1997) suggested that female attractiveness in this species may be signalled by the decalcification of the operculum covering the gonopores rather than ovary development. Jones (1997) found a correlation between ripe ovaries and female attractiveness in *I. dorsettensis*, but suggested that this evidence was purely circumstantial. Little can therefore be concluded about the nature or source of the cue indicating female attractiveness in *H. cookii* other than it originating inside the female's body rather than from her brood.

Reproductive behaviour in *H. cookii* appears to be relatively plastic. Males altered their behaviour according to the sex ratio. Copulation duration did not alter significantly when a rival male was present, with an average of 33 minutes in the standard situation and 31 minutes with a second male. However, in the presence of two females, a male copulated for a much shorter period of only approximately 6.5 minutes. This may be due to inaccurate recognition of copulation as the video equipment did not produce a clear image, and even if the pair was in the copulatory position, sperm transfer may have not necessarily occurred. However, some of these females were also used for the investigation of spermatheca capacity (Chapter 3) where they showed definite

increases in spermatheca fullness following these copulations, indicating that even in these short copulatory encounters, sperm was transferred. It would be reasonable to assume that the amount of sperm transferred is proportional to copulation duration, suggesting that perhaps the male was aware of the presence of another ripe female and spread his sperm investment accordingly. Therefore, although males may not alter the copulation duration according to female brood stage, they may do so according to sex ratio. In contrast, the presence of another male did not influence copulation duration in *Heterozius rotundifrons* (Thompson and McLay, 2005). Similarly, the duration of courtship was not affected by sex ratio in the European lobster *Homarus gammarus* (Debuse *et al*., 1999).

By reducing copulation time, male *H. cookii* may be able to control ejaculate size. This ability was seen in *Hemigrapsus sexdentatus* where males transferred larger ejaculates to larger females (Brockerhoff and McLay, 2005b). By reducing the size of the ejaculate transferred to the female, male *H. cookii* may attempt to mitigate the effects of sperm depletion by transferring less sperm to more females when possible. After transferring a large amount of sperm to a female, males may require an extended period of time to regenerate sperm and 'recharge' before adequately copulating again. *Callinectes sapidus* males took 9-20 days to fully recover to their original sperm count after mating (Kendall *et al*., 2001). In the spiny king crab *Paralithodes brevipes*, males showed little capacity to regenerate sperm and there was a significant percentage of males (42.2%) with depleted sperm reserves just after the reproductive season (Sato *et al*., 2005). However, after mating with 5 different females over a period *of* 14-33 days, male *Chionoecetes opilio* showed no significant change in the weight of ejaculate or the number of sperm cells stored by females after spawning (Sainte-Marie and Lovrich, 1994).

Mate guarding also altered according to sex ratio in *H. cookii*. Males guarded females longest in the presence of a rival male, suggesting that males protect their sperm investment more intensely when potential rival mates are in close proximity. However, the difference in guarding duration between the 1:1 (with a mean of 20 hours) and the 2 male: 1 female ratios (with a mean of almost 30 hours) was not significant when also

considering the 1 male: 2 female ratio due to the variation in the 1:1 ratio. Males may have a maximum guarding duration depending on when the brood hatches or when the costs of guarding become too high regardless of the presence of other males, or they may simply not be aware of the absence of a second male in the 1:1 situation and therefore behave no differently than in highly male biased situations. This lack of influence of sex ratio on mate guarding duration was also seen in *H. gammarus* (Debuse *et al*., 1999). In contrast, increased guarding duration was observed in a male biased sex ratio in *H. rotundifrons* (Thompson and McLay, 2005), *Menippe* spp. (Wilber, 1989), *Pagurus middendorffii* (Wada *et al*., 1999) and *Callinectes sapidus* (Jivoff and Hines, 1998).

Guarding may become too costly in female biased sex ratios. In the presence of two attractive females, guarding duration in *H. cookii* was significantly lower than in either of the other ratios. This indicates that in sex ratios dominated by females (particularly attractive females), it is more beneficial for males to reduce guarding time and invest more energy into searching and copulating with more females rather than with just one female. Holdsworth and Morse (2000) found that mate guarding duration decreased in progressively higher densities of females in the crab spider *Misumena vatia*. They suggested that the costs of guarding (lost future opportunities to mate) increase in higher female densities. Therefore, copulation and post-copulatory mate guarding in *H. cookii* may last for certain lengths of time regardless of the presence of other males, unless males have the opportunity to increase their reproductive output by mating with more attractive females with a reduced cost of searching for new mates or losing their last mate status with their current mates.

Agonistic behaviour was observed between *H. cookii* males in the presence of an attractive female. Males would spread their chelipeds toward each other, sometimes grasping onto each other. Similar behaviour was observed in *Halicarcinus varius*, *H. whitei* and *H. innominatus* (Melrose, 1975). Agonistic encounters between males were observed in *Menippe* spp. outside the female's den, resulting in several mate takeovers (Wilber, 1989). However, from the current study, it is uncertain whether male-male aggression in *H. cookii* was more intense in the presence of an attractive female than in

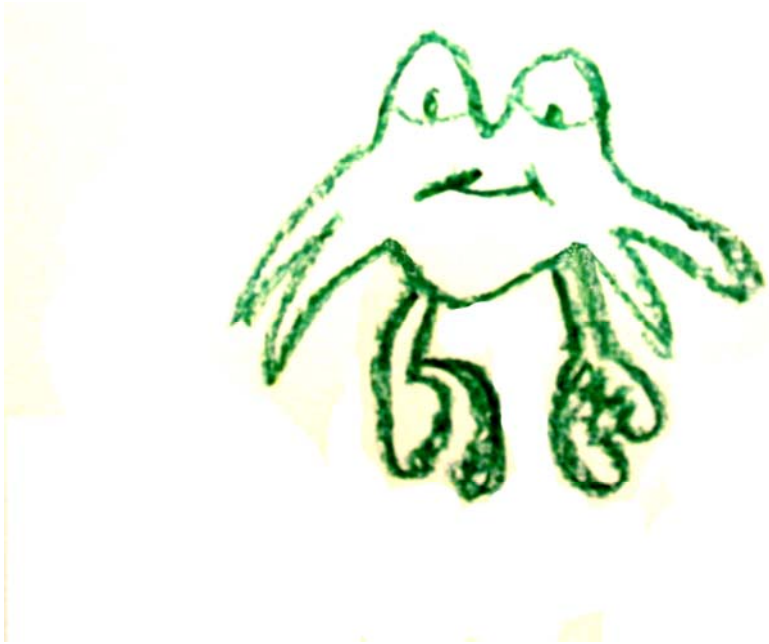
situations where no female was present. Brockerhoff and McLay (2005a) found increases in the frequency of male-male interactions when the operational sex ratio was female biased in the grapsids *Cyclograpsus lavauxi*, *Helice crassa*, *H. crenulatus* and *H. sexdentatus*. While Debuse *et al*. (1999) found that a male biased sex ratio in the lobster, *H. gammarus*, did not show an increase in male-male aggression and suggested that the males have an upper limit to the costs of inter-male competition where the risk of serious injury may prevent them from increasing aggression towards rival males when females are in short supply. However, *H. gammarus* showed female mate choice. Furthermore, Debuse *et al*. (1999) reported an increase in female competitiveness when the sex ratio was female biased. No obvious increase in aggression or competitiveness was observed in *H. cookii* females and any conclusion about the effect of sex ratio on male-male aggression requires further study.

Precocious mating is rare in *H. cookii*. Only one copulation with a juvenile female was observed, but this single copulation and the apparent attempts indicate that precocial mating does occur despite its rarity. When female ovaries develop during the penultimate instar, females are able to produce a brood of eggs immediately after the pubertal moult (Jones and Hartnoll, 1997). Although female *H. cookii* are unable to produce a brood before anecdysis, they are capable of successfully mating prior to this, and retaining sperm over their pubertal moult, after which they can almost immediately produce a brood (Chapter 3). The interest males showed in juvenile females suggest that these females were attractive to some degree despite not carrying a brood, suggesting that female attractiveness is not indicated by the brood and may provide further evidence for a signal from mature ovaries indicating female attractiveness or the use of methyl farnesoate as a development/reproduction hormone.

While most brachyuran species mate either while soft-shelled, or hard-shelled, there is evidence that some do both. This is seen in the spider crab *Inachus dorsettensis* (Jones and Hartnoll, 1997) and *Chionoecetes bairdi* (Paul, 1984). Male *H. cookii* may guard females in their penultimate instar to ensure their proximity when the female moults, allowing them to be the first to mate with the female when she does. As the female lays eggs almost immediately after moulting, the male can therefore, ensure

that his sperm is the first used to fertilize that brood. This may also explain why males may copulate with penultimate instar females. If the female can retain sperm over the pubertal moult, but does not encounter a male immediately after maturing, her first brood will be fertilized by the male she had copulated with as a juvenile. Furthermore, the guarding of a pre- and post-pubertal moult female will protect a male's sperm investment by protecting the soft female from damage or cannibalism (Leffler, 1972; Thompson, 1999). In this case, the reproductive behaviour of male *H. cookii* toward immature females close to anecdysis may resemble that of species with soft shell mating.

The mating behaviour of *H. cookii* is typical of the Hymenosomatidae (Dunnington, 1999; Hartnoll, 1969; Hosie, 2004; Lucas, 1980). Females experience a terminal, pubertal moult after which they are able to mate continuously in the hard-shell condition. Although there is evidence that copulation can occur before this moult, this appears to be rare in *H. cookii*. Paternal investment was maximized most efficiently when males showed an obvious preference for copulating with, and guarding females closest to spawning, thus reducing the risk of wasting sperm or losing future mating opportunities. The nature and source of the cue which males use to identify these most 'attractive' females remain uncertain but its presence was obvious. Copulation took approximately 30-50 minutes and the duration of guarding varied according to brood stage, lasting longest with females carrying the most developed broods. Guarding duration was longer in a male biased sex ratio and both copulation and guarding duration was significantly shorter in a female biased sex ratio, suggesting that male reproductive behaviour in *H. cookii* is flexible enough to change as the costs of guarding fluctuate.



Chapter 5

General Discussion

To begin to understand the immeasurable complexity of even just a single, isolated species, one must focus on both the basic individual features of the animal itself and the population in which it exists. The growth and population dynamics of *H. cookii* reflect the economy of energy allocation into the competing processes of growth and reproduction. In the sampled *Halicarcinus cookii* population growth and reproduction were separated so that growth primarily occurred during winter and reproduction peaked in the optimal environmental conditions during spring and summer. The most efficient means of maximizing reproductive output differs between males and females, resulting in sexual selection in *H. cookii*. Sexual selection in *H. cookii* has led to the development of various features including sexual dimorphism, where males have developed larger chelipeds and body size than females, male-male competition for females closest to spawning, female sperm storage resulting in sperm competition and last male sperm precedence and post-copulatory mate guarding as a means for a male to ensure his paternity of a brood.

Population Dynamics

In any species, the processes of growth and reproduction compete for substantial amounts of a limited energy reserve (Calow, 1978; Hartnoll, 1985). In the Brachyura, these two processes are seen to both alternate throughout the life of an individual or to occur in separate phases of the life of an individual. In species where growth is indeterminate, continuous moulting allows the individual to grow throughout their life and reproduction can occur in some or every mature instar (Hartnoll, 1985). This pattern of growth is typical of the Cancridae and Portunidae (Edwards, 1966; Edwards,

1979; Hartnoll, 1969; Hartnoll, 1985), such as *Callinectes ornatus* (Haefner, 1990), *Cancer borealis* (Elner *et al*., 1985) and the Ocypodid *Macrophthalmus hirtipes* (Jennings *et al*., 2000).

Alternatively, in species with determinate growth, growth and reproduction may be separated into different phases of the life cycle of a species. These species experience a terminal moult when growth (and with it the ability to repair damaged limbs) ceases after a certain number of instars. In some species, the terminal moult occurs when the individual becomes sexually mature, and only then can it reproduce (Hartnoll, 1969; Hartnoll, 1985). This is common in majid species, such as *Chionoecetes* spp. (Paul and Paul, 1996; Sainte-Marie and Hazel, 1992; Watson, 1970), some hymenosomatids such as *Rhynchoplax coralicola* (Gao *et al*., 1994) and particularly in the *Halicarcinus* spp. such as *H. australis* (Lucas and Hodgkin, 1970), *H. innominatus* (Dunnington, 1999) and *H. varius* (Hosie, 2004). In other species, the terminal moult does not coincide with the pubertal moult, allowing individuals to reproduce before growth ceases. This appeared to be the case in the portunids *Carcinus maenas* and *Portunus sanguinolentus* (Berrill and Arsenault, 1982; Hartnoll, 1985). Larger individuals generally have higher reproductive outputs, but growing larger requires energy that could be invested into reproduction. Both patterns are strategies to most efficiently maximize reproduction when resources are limited.

Halicarcinus cookii shows determinate growth which terminates with the pubertal moult when individuals become reproductively active. With the ability to reproduce continuously, recruitment in *H. cookii* would be expected to occur relatively consistently throughout the year, resulting in a stable population structure (Begon and Mortimer, 1986; Southwood, 1977) similar to that of *H. innominatus* at the Oaro Platform (Dunnington, 1999). With constant recruitment, population structure is largely attributable to the pattern of mortality and seasonal changes in climate. In the *H. cookii* population at First Bay and Atia Point on the Kaikoura Peninsula there was an obvious annual cycle of a somatic growth phase followed by a reproductive phase. The growth phase occurred primarily during the autumn and winter months during which the majority of new recruits were settling out of the plankton and growing through their

immature instars, becoming large enough to be detected in the sampling close to the beginning of spring when immature individuals were found in their highest proportion of the population samples. As summer approached, immature individuals developed through their pubertal moult and the population was dominated by mature individuals. By November, the vast majority of the population had matured and immature individuals made up only a small proportion of the population. Due to the terminal moult, growth in the majority of the population ceased between October and March. During the summer months, reproductive activity was at its greatest, with higher numbers of mature individuals found at this time than at any other time of the year. The numbers of mature individuals decreased in autumn as the individuals came to the end of their approximately 12-18 month life span, 6 months of which are spent as a mature adult. Such seasonal cycles were observed throughout the year despite the expectation of a relatively stable population.

With this apparent contradiction between theory and observation, external factors must be examined as potential causes of the cycling in the *H. cookii* population on the Kaikoura Peninsula. The high seasonality of the population cycles suggests that environmental factors are the primary influence on the *H. cookii* population at Kaikoura. Individuals may take longer to reach maturity in winter than in summer. As temperature decreases, physiological processes such as hormonal growth regulation and general metabolism slow down, resulting in longer inter-moult periods (Hartnoll, 1982). Assuming a fixed number of pre-pubertal instars, individuals may take longer to reach their pubertal moult in winter than in summer. Therefore, the higher percentage of juveniles observed in the winter months would be due to the increased duration of the growth phase as inter-moult intervals are extended and individuals remain juvenile for longer.

Similarly, eggs were found to take longer to develop, or failed to develop completely in cooler temperatures. No broods completed development in 10°C or below. As sea temperatures ranged from almost 18°C in January and February to 9°C in July and August, embryo and probably larval development would be restricted in the winter months. Such unsuccessful or delayed development would result in restricted

recruitment into the population over winter. Adults appeared to survive well in 10°C, suggesting that the changes in the population are more likely to be due to embryonic and larval development rather than increased mortality of the adults. The low numbers of adults present during the winter months are likely to be the last of the old generation or the first of the new one. Due to the short life span of these crabs, individuals are unlikely to survive until the following winter, creating the observed cycle in population size. The prolonged brood development would cause these individuals to produce fewer broods in their life time and therefore be disadvantaged with a lower reproductive output than those maturing in spring. This pattern of seasonal population cycling was also observed in *H. varius* (Hosie, 2004).

The varying reproductive output in *H. cookii* according to temperature has potential implications for comparisons with different populations. *H. cookii* is found throughout the east coast of New Zealand, ranging from the warmer waters in Northland to the much cooler waters of Stewart Island (McLay, 1988; Melrose, 1975). Populations along this gradient of sea temperatures may consequently show a gradient in the degree of cycling in the population. Populations in cooler waters south of Kaikoura would show a more pronounced annual cycle due to more prolonged limited reproductive output as annual temperatures in southern New Zealand average at 10-12°C (Chiswell, 1994), while in populations north of Kaikoura, where temperatures are warmer (rarely dropping below 10°C north of Wellington (Chiswell, 1994)) reproductive output would be higher compared to the Kaikoura population, resulting in a more stable population throughout the year.

Furthermore, individuals maturing in warmer temperatures are likely to be advantaged with higher reproductive output due to the ability to grow larger before reaching maturity than those in cooler temperatures. Larger *H. cookii* individuals were shown to produce larger broods, resulting in higher fecundity per brood than smaller individuals. Females were recorded to mature over a wide size range of approximately 5-9 mm CW, suggesting that environmental influences such as temperature may influence the number of juvenile instars or the percentage moult increment. The exact number of juvenile instars or even if there is a fixed number of juvenile instars in *H. cookii* is

currently unknown. Assuming the percent moult increment (PMI) remains constant throughout the growth phase it could be estimated that with a mean PMI of 18%, a female with 2 mm CW would moult approximately 10 times before reaching the mean female CW of 8.4 mm. However, PMI is likely to decrease as size increases (Richer de Forges, 1977), so conclusions relating to the number of juvenile instars in *H. cookii* require further research.

Temperature can have a parabolic effect on growth, resulting in smaller individuals at both temperature extremes and the largest individuals at intermediate temperatures (Hartnoll, 1982; Hartnoll and Bryant, 2001). This was seen in *Callinectes sapidus* where the highest percent moult increments were recorded at intermediate temperatures (Leffler, 1972). Consequently, for the *H. cookii* population at Kaikoura, the highest percent moult increment would be observed in spring compared to winter or summer. Indeed, *H. cookii* females matured at larger sizes in the early summer months than in the winter months, suggesting that they produced more eggs per brood than their winter counterparts. Therefore, due to the warmer temperatures, reproductively active individuals in summer would have an increased reproductive output than those in winter as they produce more eggs per brood and more broods in their life time. On a spatial scale, there may be a parabolic size range in *H. cookii* populations throughout New Zealand. Assuming temperatures exceed the optimum at both latitudinal extremes of the distribution of *H. cookii* in New Zealand, the lowest PMI, resulting in the smallest mature individuals would be expected in the northern-most and southern-most populations, and the highest PMI, resulting in the largest individuals would be found in the populations in between.

Sexual Selection

To increase their competitiveness in natural selection, individuals of any species are designed to maximize their reproductive fitness. Sexual selection occurs when individuals differ in reproductive success (Andersson, 1994; Andersson and Iwasa, 1996). In most species with internal fertilization, there is a conflict of interests between males and females when it comes to reproduction. Females produce large, energy-rich gametes (eggs) and maximize their reproductive output by investing energy into the

survival of offspring by provisioning their eggs with the appropriate amount of yolk. In contrast, males, who produce large quantities of energy-poor gametes (sperm) comprised almost solely of genetic material, maximize their reproductive fitness by fertilizing as many eggs as possible (Andersson, 1994; Andersson and Iwasa, 1996; Rolff, 2002).

Egg size is an important factor that affects the lifetime fecundity of a female. Hymenosomatids produce some of the smallest eggs of the Brachyura. Many hymenosomatids come close to the lower limit of egg size at which larvae remain functional (Lucas, 1980). *H. cookii* is typical of hymenosomatids, producing eggs averaging 0.025 µl in volume. By reducing egg size, a species can produce more eggs per unit expenditure of energy and material (Lucas, 1980). In species like *H. cookii* with small body size at the terminal moult, reducing egg size allows more eggs to be carried in the brood chamber, thus increasing fecundity.

Assuming egg supply is limited and sperm supply is not, this conflict between offspring quality and quantity usually results in females limiting male reproductive fitness, thus becoming a driving force for sexual selection. When sperm supply is limited, female reproductive fitness can become limited by males, as was the case in exploited populations of *Panulirus argus* and *Jasus edwardsii* due to the removal of males by fishermen (MacDiarmid and Butler, 1999). However, the population of *H. cookii* is not commercially exploited so there is no reason to assume that sperm limitation occurs in this species.

Sexual selection is driven by two main mechanisms, mate choice and intra-sexual competition (Andersson and Iwasa, 1996). Both mechanisms, alone or in concert, can result in sexual dimorphism. As males are usually limited in reproductive fitness by females, it is the males who generally develop the most pronounced secondary sexual characteristics. In species with high levels of female mate choice, males may develop highly conspicuous traits for the purpose of attracting a female, such as bright colours or ornaments. Alternatively, where there is less female mate choice, males may develop weapons for use in male-male interactions when competing to ‘win’ a female

(Andersson and Iwasa, 1996; Parker, 1970). *H. cookii* is typical of brachyurans; as males grow larger and develop much larger chelipeds in relation to body size than do females. In the Kaikoura population, males dominated the larger size classes above 12 mm CW while females were more common in the intermediate size classes. The maximum size recorded for a female was 11.51 mm, while the largest male was 12.92 mm CW. Males also showed a much stronger positive allometry in growth of both propodus length and height than did females. This obvious sexual dimorphism therefore, suggests that sexual selection is likely to occur, or has occurred in *H. cookii*.

Female choice appears to be prominent in some brachyuran species. Females selected larger males to mate with in the fiddler crab, *Uca paradussumieri* (Jaroensutasinee and Jaroensutasinee, 2003) and visual displays, where males commonly waved their major chelae in patterns designed to attract females, have been observed in many *Uca* spp. (Hazlett, 1975). In these species, female choice can be considered a driving force for sexual selection. In *H. cookii* however, there was no evidence suggesting that female choice occurs. Females did not select males according to body size, and never showed any indication of actively selecting a male to mate with. There were also no obvious visual displays exhibited by males that might serve to attract females. However, agonistic encounters between males using their chelae as weapons were observed. This suggests that the driving force of sexual selection leading to the development of large male chelipeds and probably large male body size in *H. cookii* are likely due primarily to male-male competition.

Male-male competition depends on the operational sex ratio, which includes only reproductively active males and females, rather than the population sex ratio in which all males and females are included. In brachyuran species where females are only able to mate for a short period while they have a soft integument immediately after moulting, males are highly limited by the number of receptive mates. This has been observed in the Portunidae and Cancridae in species such as *Portunus sanguinolentus* (Christofferson, 1978; Ryan, 1966) and *Carcinus maenas* (Hardege *et al*., 2002; Seifert, 1982) and *Cancer pagurus* (Edwards, 1966; Edwards, 1979). In these species, reproductively active males greatly outnumber receptive females, and therefore,

competition between males is likely to be high. Competition between males would be expected to be especially intense if and when females become receptive in synchrony, but only for a short period where females are abundant until receptive females are no longer available. This is the case for the amphipod *Corophium volutator* (McCurdy *et al*., 2000). In contrast, in *Hemigrapsus sexdentatus* all the mature females became receptive within a single month, allowing even small males a chance to mate because the large males were unable to monopolize all available females (Brockerhoff, 2002). Alternatively, if female receptivity is asynchronous, as seen in *Chionoecetes opilio* (Rondeau and Sainte-Marie, 2001), males are able to monopolize mates more easily. Competition, however, would be intense with receptive females being continuously limited (Emlen and Oring, 1977).

As *H. cookii* experiences a terminal/pubertal moult, females do not have to wait until the next moult before mating to produce a brood. Such multiparous, hard-shell mating is typical of hymenosomatids, such as *Halicarcinus innominatus* (Dunnington, 1999) and *H. varius* (Hosie, 2004) and also occurs in some majid species such as *Inachus phalangium* (Diesel, 1986; Diesel, 1988). In these species males are able to mate with females at any time, provided that females are continuously receptive, limited only by the number of females they encounter. In the *H. cookii* population, females almost consistently outnumbered males throughout the year, and particularly in the summer months of November to April. It therefore appears that the operational sex ratio is strongly biased toward females and that males need not compete for females as there are many receptive females per male. However, the sexual dimorphism and lack of female choice in *H. cookii* suggest that sexual selection does affect males. Therefore, factors other than limited mate abundance must be driving sexual selection in *H. cookii*.

Parker (1970) emphasized the importance and ubiquity of sperm competition as another, often overlooked, important mechanism of sexual selection. Sperm competition occurs when a female mates with more than one male, is able to store the sperm of multiple copulations with different males and when there is a delay between copulation and fertilization (Andersson and Iwasa, 1996; Parker, 1970; Parker, 1974). Storing viable sperm over successive copulations, moults, breeding cycles and

sometimes years indicates that a male's sperm is likely to encounter the sperm of rival males prior to fertilizing eggs (Diesel, 1991). In *H. cookii*, sperm appear to remain viable in the female spermathecae for at least the majority of the female's adult life as there was no evidence of old sperm becoming unviable. Females also appeared to be able to store enough sperm after three or four copulations to fertilize at least six of the eight broods they were estimated to be able to produce in their life time, using only 15% of a single ejaculate to fertilize each brood. Therefore, the sperm of several males may encounter each other inside the spermathecae, indicating that the level of sperm competition and consequent selection pressure for traits to increase male competitiveness in *H. cookii* is likely to be high.

Within the Decapoda, sperm storage is a unique feature (an apomorphy) of the Brachyura. The ability to store sperm is thought to be a relatively recent adaptation within the Brachyura because it is absent in the ancestral group. Since they are common in other decapod species, such as anomurans (including the *Galathea*), gonopods were almost certainly present in the brachyuran ancestor and were used to transfer spermatophores to the female sternum near the gonopores. Only brachyurans, however, have modified, tubular gonopods that act as a syringe and can enter the gonopore to deposit sperm. Internal sperm storage also requires a specialized organ inside the females body (McLay, pers. comm).

In the Hymenosomatidae, females store sperm in two spermathecae -enlarged regions of the genital duct between the vagina and the oviduct (Lucas, 1980). With spermathecae, a female can accumulate ejaculates and have a guaranteed sperm supply. In the field, *H. cookii* were often collected as solitary individuals, suggesting that encounter rates between the sexes may be low. If the number of males is limited, it may therefore be most efficient for a female to mate with any male she encounters. Furthermore, in species with sperm storage, the male's paternity of the offspring is not necessarily guaranteed simply by copulating, leading to sperm competition inside the spermatheca (McLay, pers. comm).

Sperm competition may be one of the driving forces for the development of larger male body size in *H. cookii*. Larger males have been reported to transfer more sperm and have higher reproductive output in several decapod species such as the amphipod *Gammarus pulex* (Bollache and Cezilly, 2004), the spiny lobster *Jasus edwardsii* (MacDiarmid and Butler, 1999) and the fiddler crab *Uca paradussumieri* (Jaroensutasinee and Jaroensutasinee, 2003). Likewise, larger male *H. cookii* tended to transfer more sperm to females than smaller males, giving them the ability to fertilize a greater number of eggs. Therefore, large males pass the genetic information for large body size to more offspring, which outnumber that of smaller males, leading to selection for large male body size.

Internal storage allows brachyurans to store viable sperm for relatively long periods of time. The means by which the energy-poor spermatozoa, consisting primarily of DNA material, are kept viable in brachyuran spermathecae is relatively unknown. A glycoprotein secreted from the spermathecal membrane has been suggested to maintain sperm viability in other species, such as the great lamper eel, *Amphiuma tridactylum* (Sever *et al*., 1999a), the red-spotted newt, *Notophthalmus viridescens* (Sever *et al*., 1999b) and the salamander *Eurycea Cirrigera* (Sever, 2005). However, further research is required before any similarities can be drawn between these species and brachyurans.

The intensity of sperm competition often depends on the viability of the sperm. As many decapod species have no means of storing sperm, males of these species simply present or transfer spermatophores to the female, as seen in the marine shrimps *Lysmata wurdemanni* (Bauer and Holt, 1998) and *Palaemonetes pugio* (Bauer and Abdalla, 2001) and the spiny lobster *Jasus edwardsii* (MacDiarmid and Butler, 1999). Despite a lack of evidence for average durations that spermatozoa are viable, it is reasonable to assume that without a means of storage, spermatozoa will be viable for only a short period of time. In these species the female must fertilize her eggs immediately or possibly lose the sperm. With this strategy the male can easily ensure his paternity of the female's next brood. However, in species with polyandrous females,

and where the female can store sperm, the male has no certainty of his input into egg fertilization.

The degree to which sperm mixes inside the spermathecae also influences the intensity of sperm competition. The degree of sperm mixing varies for different species. Sperm mixing can be prevented with sperm plugs, where a mucus plug is inserted following the ejaculate to prevent rivals from inseminating the female, as seen in *Telmessus cheiragonus* (Diesel, 1991; Kamio *et al*., 2000; Kamio *et al*., 2002). Alternatively, males may surround and isolate their ejaculate with pure seminal gel (no spermatozoa), preventing spermatozoa from encountering rival spermatozoa inside the spermathecae. The result is sperm layering as seen in *Halicarcinus innominatus* (Dunnington, 1999) and *Inachus phalangium* (Diesel, 1988; Diesel, 1989; Diesel, 1990). Other species show varying degrees of sperm mixing, leading to multiple paternity of a single brood, as seen in the haplogyne spider *Pycnosorus globosus* (Uhl, 1998) and the weevil *Diapreres abbreviatus* (Harari *et al*., 2002).

There appeared to be some, but little sperm mixing in *H. cookii*. If sperm layering does occur, the layers are not completely or indefinitely isolated from each other. Consequently, by increasing the amount of sperm transferred during a single copulation, a male's ejaculate can occupy more space inside the spermathecae, diluting that of rival males, so that his sperm have a higher chance of fertilizing more eggs. Therefore, larger males have a reproductive advantage over smaller males in terms of sperm competition, resulting in selection favouring increased in male size.

The structure of the spermathecae also influences sperm competition in *H. cookii*. There are two types of spermathecae in the Brachyura. In dorsal-type spermathecae the opening to the oviduct is positioned dorsally while the vagina is located ventrally on the opposite end of the spermathecae. This is common in portunid crabs such as *Carcinus maenas* and *Callinectes sapidus* (Diesel, 1991). In ventral-type spermathecae the oviduct and vagina opening are situated ventrally and close together (Diesel, 1991). This is seen in *Halicarcinus innominatus* (Dunnington, 1999), *H. varius* (Hosie, 2004), *Chionoecetes opilio* (Beninger *et al*., 1988), *Inachus phalangium* (Diesel, 1989), and in

the families Calappidae, Geryonidae, Leucosiidae and Corystidae (Diesel, 1991). *H. cookii* also has ventral-type spermathecae. Due to this structure, the sperm located closest to the entrance of the spermathecae (the last sperm transferred) is the first used to fertilize eggs. This last male sperm precedence has significant implications for the reproductive strategies of male *H. cookii*.

To maximize their reproductive output, males may have two strategies to ensure their sperm is used to fertilize eggs according to whether the female is primiparous or multiparous. Precocial mating may indicate a reproductive strategy for primiparous females. One copulation with a juvenile female was observed and eight females were observed to produce a fertilized brood despite having been isolated from males as juveniles. Males may copulate with a juvenile female to ensure their sperm is used to fertilize that female's first brood as she is unlikely to encounter another male before laying a brood in the first few hours immediately following anecdysis. However, males more commonly copulated with multiparous females. With the last male sperm precedence in *H. cookii*, males would most efficiently mate if they can ensure that they are the last to copulate with the female. Males must then be able to discriminate between females who are likely to re-mate before laying a new brood and those with whom they are likely to be the last to mate.

Ovary development was shown to coincide with brood development in *H. cookii*. This implies that a female carrying a well developed brood close to spawning is also closer to laying a new brood than females carrying less developed broods. Therefore, males are most likely to maximize paternity of a brood if they copulate with a female about to spawn. Furthermore, if sperm mixing inside the spermathecae increases over time, it can be minimized if the latest ejaculate is used immediately. Consequently, females carrying broods about to hatch (stage 5) are the most efficient and therefore attractive females for males to mate with because they are least likely to re-mate before laying a new brood.

Males must be able to identify attractive females to most efficiently mate. There is evidence for the use of a moulting hormone such as crustecdysone doubling as a

mating pheromone and used by males to detect female receptivity in species where moulting and mating are linked (Christofferson, 1978; Hardege *et al*., 2002; Kamio *et al*., 2000; Kamio *et al*., 2002; Kittredge *et al*., 1971; Thompson, 1999). However, the ability of female *H. cookii* to mate in the hard-shell condition rules out crustecdysone as a possible pheromone signalling female attractiveness to males. Male *H. cookii* reacted in the same way to females according to brood stage when the brood had been removed, indicating that the cue signalling female attractiveness did not come from the brood. Furthermore, males were attracted to juvenile females, who cannot produce a brood. This suggests that the use of the development and reproduction hormone found in many arthropod species, methyl farnesoate (Laufer and Ahl, 1995; Laufer *et al*., 1992; Laufer *et al*., 2002; Laufer *et al*., 1987) may also be used in *H. cookii*, or that males may be able to detect the development of the ovaries as they coincide with brood development. Furthermore males were not attracted to moulting by immature females, supporting the latter mechanism.

The primary selective force driving sexual dimorphism in *H. cookii* may be male-male competition for limited attractive mates. Although mature females almost consistently outnumbered males throughout the year, the sex ratio including females only carrying stage 5 brood was consistently and significantly male biased. Despite the ability of *H. cookii* females to mate at any time in the hard-shell condition, males must compete for the small proportion of mature females of whose next brood they can ensure maximum paternity. The male reproductive behaviour observed in *H. cookii* lends further support for this selective pressure.

Male *H. cookii* showed a significant preference for guarding stage 5 females and for guarding them longer than any other stage. Guarding a female after copulation can be an effective strategy for a male to protect his reproductive investment (Diesel, 1991; Smith, 1984). Male *H. cookii* can prevent females copulating with rival males through post-copulatory mate guarding. However, guarding is very costly in terms of energetic input, lost feeding time and, primarily, loss of future mating opportunities (Holdsworth and Morse, 2000). Therefore, it is most efficient to preferentially guard females requiring the least time investment. On many occasions the brood of the guarded stage

5 female hatched, indicating that the male had successfully ensured his last-mate status and would fertilize the majority, if not all of the eggs in the female's next brood, which she laid almost immediately after spawning.

Mate guarding in *H. cookii* appears to be a relatively plastic behaviour. Males decreased both copulation and guarding duration when there was opportunity to mate with two stage 5 females without the presence of other males. In an attractive female biased sex ratio, it becomes too costly (in terms of lost future mating opportunities) for males to invest long periods of time into a single female (Holdsworth and Morse, 2000). In these cases, with fewer rival males to compete with, males can increase their reproductive fitness by mating with as many females as possible. Some species show increases in copulation and guarding duration in a male biased sex ratio, such as *Halicarcinus rotundifrons* (Thompson and McLay, 2005), *Menippe* spp. (Wilber, 1989) *Pagurus middendorffii* (Wada *et al*., 1999) and *Callinectes sapidus* (Jivoff and Hines, 1998). It is more efficient for males of these species to invest more time into each female due to the higher risk of loosing paternity to rival males. Although guarding duration was greater in a male biased sex ratio in *H. cookii*, it was not significantly greater when all three sex ratios were considered, suggesting that either males were unaware of the presence, or absence of a rival male, or that males generally guard until a threshold is crossed where the costs of guarding become too great.

In conclusion, the many facets of the ecology and biology of *Halicarcinus cookii* are intimately interconnected, making this small, ubiquitous New Zealand crab a complex and intricate species. The inherent pressure to maximize individual reproductive output is the principal influence on the reproductive strategies of *H. cookii*. Individual reproductive output in this species varies according to season; peaking in the warmer months and decreasing in the cooler months. The terminal moult and the predominance of spring maturation in the population allow females to produce eggs continuously throughout the peak reproductive season, thus maximizing the number of offspring produced in this species that has such a short life span. Sexual dimorphism is obvious in *H. cookii*, indicating the pressure of sexual selection. The lack of female choice suggests male-male competition is the primary cause of the observed sexual

dimorphism. Larger males have a reproductive advantage over smaller males through both competitions for mates and sperm competition. Although mature females are not limiting, the most optimal ones for males to mate with are. The structure of the spermathecae in *H. cookii* results in last male sperm precedence, implying that males compete to be the last to mate with a female before a new brood is laid. Although the means of identification remains unknown, males preferentially mate with females close to spawning as they are also closest to laying a new brood. Through post-copulatory mate guarding a male can further protect his last-mate status and the duration of this behaviour can alter according to the varying costs and benefits related to the operational sex ratio.

Ali MH, Salman SD. 1998. Production of the crab Elamenopsis kempi (Hymenosomatidae) in the Garmat-Ali region, Basrah, Iraq. Marine Ecology-Pubblicazioni Della Stazione Zoologica Di Napoli I 19(1):67-75.

Ali MH, Salman SD, Aladhub AY. 1995. Population dynamics of the hymenosomatid crab *Elamenopsis kempi* in a brackish subtidal region of Basra, Iraq. Scientia Marina 59(1):1-13.

Andersson M. 1994. Sexual Selection. Krebs JR, Clutton-Brock T, editors. Princeton: Princeton University Press.

Andersson M, Iwasa Y. 1996. Sexual selection. Trends in Ecology and Evolution 11(2):53-58.

Anilkumar G, Sudha K, Subramoniam T. 1999. Spermatophore transfer and sperm structure in the brachyuran crab *Metograpsus messor* (Decapoda: Grapsidae). Journal of Crustacean Biology 19(2):361-370.

Bamber SD, Naylor E. 1997. Sites of release of putative sex pheromone and sexual behaviour in female *Carcinus maenas* (Crustacea: Decapoda). Estuarine, Coastal and Shelf Science 44:194-202.

Bauer RT, Abdalla J. 2001. Male mating tactics in the shrimp *Palaemonetes pugio* (Decapoda, Caridea): precopulatory mate guarding vs. pure searching. Ethology 107:185-199.

Bauer RT, Holt G. 1998. Simultaneous hermaphroditism in the marine shrimp *Lysmata wurdemanni*: an undescribed sexual system in the decopod Crustacea. Marine Biology 132(223-235).

Begon M, Mortimer M. 1986. Population Ecology: a unified study of animals and plants. second ed. Oxford: Blackwell Scientific Publications. 220 p.

Beninger PG, Elner RW, Foyle TP, Odense PH. 1988. Functional anatomy of the male reproductive system and the female spermatheca in the snow crab *Chionoecetes opilio* (O. Fabricius) (Decapoda: Majidae) and a hypothesis for fertilisation. Journal of Crustacean Biology 8(3):322-332.

Beninger PG, Lanteigne C, Elner RW. 1993. Reproductive processes revealed by spermatophore dehiscence experiments and by histology, ultrastructure, and histochemistry of the female reproductive system in the snow crab *Chionoecetes opilio* (O. Fabricius). Journal of Crustacean Biology 13(1):1-16.

Berrill M, Arsenault M. 1982. Mating behaviour of the green shore crab *Carcinus maenas.* Bulletin of Marine Science 32(2):632-638.

Bollache L, Cezilly F. 2004. Sexual Selection on male body size and assortative pairing in *Gammarus pulex* (Crustacea: Amphipoda): field surveys and laboratory experiments. Journal of Zoology 264:135-141.

Brockerhoff AM. 2002. Comparative studies of the reproductive strategies of New Zealand grapsid crabs (Brachyura: Grapsidae) and the effects of parasites on their reproductive success. [PhD]. Christchurch: University of Canterbury. 177 p.

Brockerhoff AM, McLay CL. 2005a. Comparative analysis of the mating strategies in grapsid crabs with special reference to two common intertidal crabs, *Cyclograpsus lavauxi* and *Helice crassa* from New Zealand. Journal of Crustacean Biology 25(3):507-520.

Brockerhoff AM, McLay CL. 2005b. Mating behaviour, female receptivity and male-male competition in the intertidal crab *Hemigrapsus sexdentatus* (Brachyura: Grapsidae). Marine Ecology Progress Series 290:179-191.

Broekhuysen GJ. 1955. The breeding and growth of *Hymenosoma orbiculare* Desm. (Crustacea, Brachyura). Annals of the South African Museum 41:313-343.

Bryant AD, Hartnoll RG. 1995. Reproductive investment in two spider crabs with different breeding startegies. Journal of Experimental Marine Biology and Ecology 188:261-275.

Calow P. 1978. The cost of reproduction - a physiological approach. Biological Review 54:23-40.

Carlisle DB. 1957. On the hormonal inhibition of moulting in decapod Crustacea; II The terminal anecdysis in crabs. Journal of the marine Biological Association of the UK 36(2):291-307.

Carvalho N, Alfonso P, Santos R. 2003. The haremic mating system and mate choice in the wide-eyed flounder, *Bothus podas*. Environmental Biology of Fishes 66(3):249-258.

Cheung TS. 1968. Transmoult retention of sperm in the female stone crab *Menippe mercenaria* (Say). Crustaceana 15(117-120).

Chiswell SM. 1994. Variability in sea surface temperature around New Zealand from AVHRR images. New Zealand Journal of Marine and Freshwater Reasearch 28:179-192.

Christofferson JP. 1978. Evidence for the controlled release of a crustacean sex pheromone. Journal of Chemical Ecology 4(6):633-639.

Christy JH. 1987. Competitive mating, mate choice and mating associations of brachyuran crabs. Bulletin of Marine Science 41(2):177-191.

Chuang CTN, Ng PKL. 1994. The ecology and biology of Southeast-Asian false spider crabs (Crustacea, Decapoda, Brachyura, Hymenosomatidae). Hydrobiologia 285(1-3):85-92.

Claxton WT, Govinid CK, Elner RW. 1994. Chela function, morphometric maturity and the mating embrace in male snow crab, *Chionoecetes opilio*. Canadian Journal of Fisheries and Aquatic Sciences 51:1110--1118.

Colby D, Fonseca M. 1984. Population dynamics, spatial dispersion and somatic growth of the sand fiddler crab *Uca pugilator.* Marine Ecology Progress Series 16(3):269-279.

Comeau M, Conan GY. 1992. Morphometry and gonad maturity of male snow crab, *Chionoecetes opilio*. Canadian Journal of Fisheries and Aquatic Sciences 49:2460-2468.

Conan GY, Comeau M. 1986. Functional maturity and terminal molt of the male snow crab, *Chionoecetes opilio*. Canadian Journal of Fisheries and Aquatic Sciences 43:1710-1719.

Danielsson I. 1998. Mechanisms of sperm competition in insects. Annales Zoologici Fennici 35:241-257.

Debuse VJ, Addison JT, Reynolds JD. 1999. The effects of sex ratio on sexual competition in the European lobster. Animal Behaviour 58(5):973-981.

DeGoursey RE, Auster PJ. 1992. A mating aggregation of the spider crab (*Libinia emarginata*). Journal of Northwest Atlantic Fishery Science 13:77-82.

Diaz H, Eloy Conde J. 1989. Population dynamics and life history of the mangrove crab *Aratus pisonii* (Brachyura, Grapsidae) in a marine environment. Bulletin of Marine Science 45(1):148-163.

Diesel R. 1986. Optimal mate searching strategy in the symbiotic spider crab *Inachus phalangium* (Decapoda). Ethology 72:311-328.

Diesel R. 1988a. Discrete storage of multiple - mating sperm in the spider crab *Inachus phalangium*. Naturwissenschaffen 75:148-149.

Diesel R. 1988b. Male-female association in the spider crab *Inachus phalangium*: the influence of female reproductive stage and size. Journal of Crustacean Biology 8(1):63-69.

Diesel R. 1989. Structure and function of the reproductive system of the symbiotic spider crab *Inachus phalangium* (Decapoda: Majidae): observations on sperm

transfer sperm storage and spawning. Journal of Crustacean Biology 9(2):266-277.

Diesel R. 1990. Sperm competition and reproductive success in the decapod *Inachus phalangium* (Majidae): a male ghost spider crab that seals off rivals' sperm. The Zoological Society of London 220:213-223.

Diesel R. 1991. Sperm competition and the evolution of mating behaviour in Brachyura, with special reference to spider crabs (Decapoda, Majidae). In: Bauer RT, Martin JW, editors. Crustacean sexual biology. New York: Colombia University Press.

Donaldson WE, Adams AE. 1989. Ethogram of behaviour with emphasis on mating for the tanner crab *Chionoecetes bairdi* Rathbun. Journal of Crustacean Biology 9:37-53.

Dunnington MJ. 1999. The reproductive strategies of the pill-box crab *Halicarcinus innominatus* Richardson, 1949. Christchurch: University of Canterbury. 140 p.

Edwards E. 1966. Mating behaviour in the european edible crab (*Cancer pagurus* L.). Crustaceana 10:23-30.

Edwards E. 1979. The edible crab and its fishery in British waters. Farnham, Surrey: Fishing News Books Ltd. 142 p.

Efford IE. 1967. Neoteny in sand crabs of the genus *Emerita* (Anomura, Hippidae). Crustaceana 13:81-93.

Elner RW, Beninger PG. 1995. Multiple reproductive startegies in snow crab, *Chionoecetes opilio*: Physiological pathways and behavioural plasticity. Journal of Experimental Marine Biology and Ecology 193:93-112.

Elner RW, Gass C, A., Campbell A. 1985. Mating behaviour of the Jonah crab, *Cancer borealis* Stimpsom (Decapoda, Brachyura). Crustaceana 48(1):35-39.

Emlen ST, Oring LW. 1977. Ecology, sexual selection, and the evolution of mating systems. Science 197:245-223.

Fukui Y. 1993. Timing of copulation in the molting and reproductive cycles in a grapsid crab *Gaetice depressus* (Crustacea: Brachyura). Marine Biology 117:221-226.

Furota T. 1996. Lifecycle studies on the introduced spider crab *Pyromaia tuberculata* (Lockington) (Brachyura: Majidae). I Egg and larval stages. Journal of Crustacean Biology 16(1):71-76.

Gao T, Tsuchida S, Watanabe S. 1994. Growth and reproduction of *Rhynchoplax coralicola* Rathbun (Brachyura: Hymenosomatidae). Crustacean Research 23:108-116.

Gardner C, Williams H. 2002. Maturation in the male giant crab, *Pseudocarcinus*, and the potential for sperm limitaion in the Tasmanian fishery. Marine and Freshwater research 53:661-667.

Gleeson RA. 1991. Intrinsic factors mediating pheromone communication in the blue crab *Callinectes sapidus.* In: Bauer RT, Martin JW, editors. Crustacean Sexual Biology. New York: Columbia University Press. p 17-32.

Goshima S, Kawashima T, Wada S. 1998. Mate choice by males of the hermit crab *Pagurus filholi*: Do males assess ripeness and/or fecundity of females? Ecological Research 13:151-161.

Haefner PA. 1985. Morphometry, reproduction, diet and epizoites of *Ovalipes stephensoni* Williams, 1976 (Decapoda, Brachyura). Journal of Crustacean Biology 5(4):658-672.

Haefner PA. 1990. Morphometry and size at maturity of *Callinectes ornatus* (Brachyura, Portunidae) in Bermuda. Bulletin of Marine Science 46(2):274-286.

Hankin DG, Diamond N, Mohr MS, Ianelli J. 1989. Growth and reproductive dynamics of adult female dungeness crabs (*Cancer magister*) in northern California. Journal du Conseil 46(1):94-108.

Harari AR, Landolt PJ, O'Brien CW, Brockmann HJ. 2002. Prolonged mate guarding and sperm competition in the weevil Diaprepes abbreviatus. Behavioral Ecology 14:89-86.

Hardege JD, Jennings A, Hayden D, Müller CT, Pascoe D, Bentley MG, Clare AS. 2002. Novel behavioural assay and partial purification of a female-derived sex pheromone on *Carcinus maenas*. Marine Ecology Progress Series 244:179-189.

Hartnoll RG. 1965. The biology of spider crabs: a comparison of British and Jamaican species. Crustaceana 9:1-16.

Hartnoll RG. 1969. Mating in the Brachyura. Crustaceana 16:161-181.

Hartnoll RG. 1978. The determination of relative growth in Crustacea. Crustaceana 34(3):281-293.

Hartnoll RG. 1982. Growth. In: Abele LG, editor. The Biology of Crustacea: Embryology, Morphology and Genetics. New York: Academic Press. p 111-196.

Hartnoll RG. 1985. Growth, sexual maturity and reproductive output. In: Wenner AM, editor. Factors in Adult Growth. Rotterdam: A.A. Balkema.

Hartnoll RG, Bryant AD. 2001. Growth to maturity of juveniles of the spider crabs *Hyas coarctatus* Leach and *Inachus dorsettensis* (Pennant) (Brachyura: Majidae). Journal of Experimental Marine Biology and Ecology 263(2):143-158.

Hazlett B. 1975. Ethological Analyses of reproductive behaivour in Marine Crustacea. Pubbl Staz Zool Napoli 39(677-695).

Hines AH. 1982a. Allometric constraints and variables of reproductive effort in brachyuran crabs. Marine Biology 69:309-320.

Hines AH. 1991. Fecundity and reproductive output in nine species of *Cancer* crabs (Crustacea, Brachyura, Cancridae). Canadian Journal of Fisheries and Aquatic Sciences 48:267-275.

Hines AH. 1992. Fecundity and reproductive output in nine species of cancer crabs (Crustacea, Brachyura, Cancridae). Canadian Journal of Fisheries and Aquatic Sciences 48:267-275.

Hinsch GW. 1968. Reproductive behaviour in the spider crab, *Libinia emarginata* (L). Biological Bulletin 135:273-278.

Holdsworth AR, Morse DH. 2000. Mate guarding and aggression by the crab spider Misumena vatia in relation to female reproductive status and sex ratio. American Midland Naturalist 143(1):201-211.

Horn R. Harms J. 1988. Larval development of *Halicarcinus varius* (Decapoda: Hymenosomatidae). New Zealand Journal of Marine and Freshwater Reasearch 22(1):1-8.

Hosie AM. 2004. The reproductive ecology of *Halicarcinus varius* (Brachyura: Hymenosomatidae) Dana, 1851: University of Canterbury.

Jansen KP. 1974. Ecological studies on intertidal Sphaeromatidae (Isopoda: Flabellifera). Marine Biology 11:262-285.

Jaroensutasinee M, Jaroensutasinee K. 2003. Male body size influences female choice and male-male competition in the fiddler crab *Uca paradussumieri* Bott, 1973 (Decapoda, Brachyura, Ocypodidae). Crustaceana 76(2):177-186.

Jennings AC, McLay CL, Brockerhoff AM. 2000. Mating behaviour of *Macrophthalmus hirtipes* (Brachyura: Ocypodidae). Marine Biology 137:267-278.

Jensen PC, Orensanz JM, Armstrong DA. 1996. Structure of the female reproductive tract in the dungeness crab (*Cancer magister*) and implications for the mating system. Biological Bulletin 190:336-349.

Jewett SC, Sloan NA, Somerton DA. 1985. Size at sexual maturity and fecundity of the fjord-dwelling golden king crab, *Lithodes aequispina* Benedict, from northern British Columbia. Journal of Crustacean Biology 5(3):377-385.

Jirotkul M. 2000. Operational sex ratio influences the opportunity for sexual selection in guppies. Journal of Fish Biology 56(3):739-741.

Jivoff P. 1997a. The relative roles of predation and sperm competition on the duration of the post copulatory association between the sexes in the blue crab, *Callinectes sapidus.* Behavioural Ecology and Sociobiology 40:175-185.

Jivoff P. 1997b. Sexual competition among male blue crab, *Callinectes sapidus*. Biological Bulletin 193:368-380.

Jivoff P. 2003a. A review of male mating success in the blue crab, *Callinectes sapidus*, in reference to the potential for fisheries-induced sperm limitation. Bulletin of Marine Science 72(2):273-286.

Jivoff P. 2003b. Summary session paper Blue Crab Symposium 2000 - Reproduction and embryonic development. Bulletin of Marine Science 72(2):265-272.

Jivoff P, Hines AH. 1998. Female behaviour, sexual competition and mate guarding in the blue crab, *Callinectes sapidus*. Animal Behaviour 55:589-603.

Jones DR, Hartnoll RG. 1997. Mate selection and mating behaviour in spider crabs. Estaurine, Coastal and Shelf Science 44:185-193.

Jormalainen V. 1998. Precopulatory mate guarding in crustaceans: male competitive strategy and intersexual conflict. The quarterly review of biology 73(3):275-304.

Jormalainen V, Merilaita S. 1995. Female resistance and duration of mate guarding in three aquatic peracarids (Crustacea). Behavioral Ecology and Sociobiology 36(1):43-48.

Kamio M, Matsunaga S, Fusetani N. 2000. Studies on sex pheromones of the helmet crab, *Telmessus cheiragonous* 1. An assay based on precopulatory mate guarding. Zoological Science 17:731-733.

Kamio M, Matsunaga S, Fusetani N. 2002. Copulation pheromone in the crab *Telmessus cheiragonus* (Brachyura: Decapoda). Marine Ecology Progress Series 234:183-190.

Kendall MS, Wolcott DS, Wolcott TG, Hines AH. 2001. Reproductive potential of individual male blue crabs, *Callinectes sapidus*, in a fished population: Depletion and recovery of sperm number and seminal fluid. Canadian Journal of Fisheries and Aquatic Sciences 58(6):1168-1178.

Kendall MS, Wolcott DS, Wolcott TG, Hines AH. 2002. Influence of male sperm size and mating history on sperm content of ejaculates of the blue crab *Callinectes sapidus*. Marine Ecology Progress Series 230:235-240.

Kittredge JS, Terry M, Takahashi FT. 1971. Sex pheromone activity of the moulting hormone, crustecdysone, on males crabs (*Pachygrapsus crassopes*, *Cancer antennarius* and *C. anthonyi*). Fishery Bulletin 69(2):337-343.

Knudsen JW. 1960. Reproduction, life history, and larvae ecology of the California Xanthidae, the pebble crabs. Pacific Science 14:3-17.

Koga T, Henmi Y, Murai M. 1993. Sperm competition and the assurance of underground copulation in the sand-bubbler crab *Scopimera globosa* (Brachyura: Ocypodidae). Journal of Crustacean Biology 13(1):134-137.

Laufer H, Ahl JSB. 1995. Mating behaviour and methyl farnesoate levels in male morphotypes of the spider crabs, *Libinia emarginata* (Leach). Journal of Experimental Marine Biology and Ecology 193:15-20.

Laufer H, Ahl JSB, Homola E. 1992. Methyl farnesoate appears to be a crustacean reproductive hormone. Invertebrate Reproduction and Development 22(1-3):17-20.

Laufer H, Ahl JSB, Rotllant G, Baclaski B. 2002. Evidence that ecdysteroids and methyl farnesoate control allometric growth and differentiation in a crustacean. Insect Biochemistry and Molecular Biology 32:205-210.

Laufer H, Borst D, Baker FC, Carrasco C, Sinkus M, Reuter CC, Tsai LW, Schooley DA. 1987. Identification of a juvenile hormone-like compound in a crustacean. Science 235:202-205.

Leffler CW. 1972. Effects of temperature on the growth and metabolic rate of juvenile blue crabs, *Callinectes sapidus*, in the laboratory. Marine Biology 14(2):104-110.

Leigh AM. 1970. Sex ratio and the differential mortality between the sexes. The American Naturalist 104(936):205-210.

Lewis EG. 1977. Relative growth and sexual maturity of *Bathynectes superbus* (Costa) (Decapoda: Portunidae). Journal of Natural History 11:629-643.

Lucas JS. 1980. Spider crabs of the family Hymenosomatidae (Crustacea; Brachyura) with particular reference to australian species: systematics and biology. Records of the Australian Museum 33(4):148-247.

Lucas JS, Hodgkin EP. 1970a. Growth and reproduction of *Halicarcinus australis* (Haswell) (Crustacea, Brachyura) in the Swan Estuary, Western Australia II.

larval stages. Australian Journal of Marine and Freshwater Research 20(2):163-173.

Lucas JS, Hodgkin EP. 1970b. Growth and reproduction of *Halicarcinus australis* (Haswell) (Crustacea, Brachyura) in the Swan Estuary, Western Australia, I. Crab instars. Australian Journal of Marine and Freshwater Research 21:149-162.

MacDiarmid AB, Butler MJ. 1999. Sperm economy and limitation in New Zealand spiny lobster. Behavioral Ecology and Sociobiology 46(1):14-24.

Margolies DC, Collins RD. 1994. Chemically-mediated pre-mating behavior in two tetranychid species. Experimental & Applied Acarology 18(8):493-501.

Mathews LM. 2002. Tests of the mate-guarding hypothesis for social monogamy: does population density, sex ratio, or female synchrony affect behaviour of male snapping shrimp (*Alpheus angulatus*)? Behavioural Ecology and Sociobiology 51:426-432.

McCleese DW, Spraggins RL, Bose AK, Praminik BN. 1977. Chemical and behavioural studies of the sex attractant of the lobster (*Homarus americanus*). Marine Behaviour and Physiology 4:219-232.

McCurdy DG, Boates JS, Forbes MR. 2000. Reproductive synchrony in the intertidal amphipod *Corophium volutator*. Oikos 88:301-308.

McLay CL. 1988. Crabs of New Zealand. Auckland: University of Auckland.

Melrose MJ. 1975. The Marine Fauna of New Zealand: Family Hymenosomatidae (Crustacea, Decapoda, Brachyura). Wellington: New Zealand Department of Scientific and Industrial Research. 123 p.

Menzies HP. 1988. Growth Reproduction and Diet of the Pill-Box Crab *Halicarcinus innominatus* Richardson, 1949 (Brachyura: Hymenosomatidae. Christchurch: University of Canterbury. 86 p.

Moriyasu M, Comeau M. 1996. Grasping behaviour of male snow crab *Chionoecetes opilio* (O. Fabricius, 1788) (Decapoda, Majidae). Crustaceana 69(2):211-222.

Negreiros-Fransozo ML, Colpo KD, Costa TM. 2003. Allometric Growth in the Fiddler Crab *Uca thayeri* (Brachyura, Ocypodidae) From a Subtropicla Mangrove. Journal of Crustacean Biology 23(2):273-279.

Ottaway JR. 1976. Inshore sea temperatures at Kaikoura, New Zealand, 1973-1975. Mauri Ora.

Parker GA. 1970. Sperm composition and its evolutionary consequences in the insects. Biological Review 45:525-567.

Parker GA. 1974. Courtship persistence and female-guarding as male time investment strategies. Behaviour 48:157-184.

Paul AJ. 1984. Mating frequency and viability of stored sperm in the tanner crab *Chionoecetes bairdi* (Decapoda, Majidae). Journal of Crustacean Biology 4(3):375-381.

Paul AJ, Paul JM. 1992. Second clutch viability of *Chionoecetes bairdi* (Decapoda, Majidae) inseminated only at the maturity molt. Journal of Crustacean Biology 12(3):438-441.

Paul AJ, Paul JM. 1996. Observations on mating of multiparous *Chionoecetes Bairdi* Rathbun (Decapoda: Majidae) held with different sizes of males and one-clawed males. Journal of Crustacean Biology 16(2):295-299.

Richer de Forges B. 1977. Étude du Crabe des Iles Kerguelen: *Halicarcinus planatus* (Fabricius). Comité National Français Des Recherches Antarctiques 42:71-133.

Rolff J. 2002. Bateman's principle and immunity. Proceedings of the Royal Society B, Biological Sciences 269(1493):867.

Rondeau A, Sainte-Marie B. 2001. Variable mate guarding time and sperm allocation by male snow crabs (*Chionoecetes opilio*) in response to sexual competition, and their impact on the mating success of females. Biology Bulletin 201:204-217.

Ryan EP. 1966. Pheromone: evidence in decapod crustacea. Science 151:340-341.

Sainte-Marie B. 1993. Reproductive cycle and fecundity of primiparous and multiparous female snow crab *Chionoecetes opilio*, in the Northwest Gulf of Saint Lawrence. Canadian Journal of Fisheries and Aquatic Sciences 50:2147-2156.

Sainte-Marie B, Hazel F. 1992. Moulting and mating in snow crabs, *Chionoecetes opilio.* Canadian Journal of Fisheries and Aquatic Sciences 49:12382-11293.

Sainte-Marie B, Lovrich GA. 1994. Delivery and storage of sperm at first mating of female *Chionoecetes opilio* (Brachyura: Majidae) in relation to size and morphometric maturity of male parent. Journal of Crustacean Biology 14(3):508-521.

Sainte-Marie G, Sainte-Marie B. 1999. Reproductive products in the adult snow crab (*Chionoecetes opilio*). II. Multiple types of sperm cells and of spermatophores in the spermathecae of mated females. Canadian Journal of Zoology 77(3):451-462.

Sainte-Marie B, Sevigny J, Carpentier M. 2002. Interannual variability of sperm reserves and fecundity of primiparous of the snow crab (*Chionoecetes opilio*) in relation to sex ratio. Canadian Journal of Fisheries and Aquatic Sciences 59:1932-1940.

Sainte-Marie B, Urbani N, Sevigny JM, Hazel F, Kuhnlein U. 1999. Multiple choice criteria and the dynamics of assortative mating during the first breeding period season of female snow crab *Chionoecetes opilio* (Brachyura, Majidae). Marine Ecology Progress Series 181:141-153.

Sainte-Marie G, Sainte-Marie B, Sevigny J. 2000. Ejaculate storage patterns and the site of fertilisation in female snow crabs (*Chionoecetes opilio;* Brachyura, Majidae). Canadian Journal of Zoology 78(1902-1917).

Salmon M. 1967. Coastal distribution, display and sound production by Florida fiddler crabs. Animal Behaviour 15:449-459.

Sato T, Ashidate M, Wada S, Goshima S. 2005. Effects of male mating frequency and male size on ejaculate size and reproductive success of female spiny king crads *Paralithodes brevipes*. Marine Ecology Progress Series 296:251-262.

Seifert P. 1982. Studies on the sex pheromone of the shore crab, *Carcinus maenas*, with special regard to ecdysone excretion. Ophelia 21(2):147-158.

Sever DM. 2005. Sperm storage and degradation in the spermathecae of the salamander *Eurycea Cirrigera*. Journal of Morphology 210(1):71-84.

Sever DM, Doody JS, Reddish CA, Wenner MM, Church DR. 1999a. Sperm storage in Spermathecae of the great lamper eel, *Amphiuma tridactylum* (Caudata: Amphiumidae). Journal of Morphology 230(1):79-97.

Sever DM, Rania LC, Krenz JD. 1999b. Annual cycle of sperm storage in spermathecae of the red-spotted newt, *Notophthalmus viridescens* (Amphibia: Salamandridae). Journal of Morphology 227(2):155-170.

Shields JD. 1991. The reproductive ecology and fecundity of *Cancer* crabs. In: Wenner A, Kuris A, editors. Crustacean egg production. Rotterdam: A.A. Balkema. p 193-213.

Smith RL. 1984. Sperm competition and the evolution of animal mating systems.

Somers KM. 1991. Characterizing size specific fecundity in crustaceans. In: Wenner AM, Kuris A, editors. Crustacean Egg Production. Rotterdam: A.A. Balkema. p 357-378.

Southwood TRE. 1977. Habitat, the templet for ecological Strategies? Journal of Animal Ecology 46:337-365.

Stevens BG, Donaldson WE, Haaga JA, Munk JE. 1993. Morphometry and maturity of paired tanner crabs, *Chionoecetes bairdi*, from shallow- and deepwater environments. Canadian Journal of Fisheries and Aquatic Sciences 50:1504-1516.

Stevens BG, Haaga JA, Donaldson WE. 1994. Aggregative mating of tanner crabs, *Chionoecetes bairdi.* Canadian Journal of Fisheries and Aquatic Sciences 51(6):1273-1280.

Swiney KM, Shirley TC. 2001. Gonad development of southeastern Alaskan dungeness crab, Cancer magister, under laboratory conditions. Journal of Crustacean Biology 21(4):897-904.

Thompson GA. 1999. Mating Behaviour and the Reproductive Ecology of the Big-Handed Crab, *Heterozius rotundifrons* A. Milne Edwards, 1867. Christchurch: University of Canterbury. 180 p.

Thompson GA, McLay CL. 2005. Mating behaviour of Heterozius rotundifrons (Crustacea: Brachyura: Belliidae): is it a hard or soft shell mater? Marine and Freshwater research 55(8):1107-1116.

Tsuchida S, Watanabe S. 1997. Growth and reproduction of the grapsid crab *Plagusia dentipes* (Decapoda: Brachyura). Journal of Crustacean Biology 17(1):90-97.

Uhl G. 1998. Mating behaviour in the cellar spider, Pholcus phalangioides, indicates sperm mixing. Animal Behaviour 56(5):1155-1159.

Wada S, Tanaka K, Goshima S. 1999. Precopulatory mate guarding in the hermit crab *Pagurus middendorffii* (Brandt) (Decapoda: Paguridae): effects of population parameters on male guarding duration. Journal of Experimental Marine Biology and Ecology 239:289-298.

Walker K. 1969. The Ecology and Distribution of *Halicarcinus lacustris* (Brachyura: Hymenosomatidae) in Australian inland waters. Australian Journal of Marine and Freshwater Research 20:163-173.

Watson J. 1970. Mating behaviour in the spider crab, *Chionoecetes opilio.* Journal of the Fisheries Research Board of Canada 27(9):1607-1616.

Wear RG. 1974. Incubation in British decapod crustacea, and the effects of temperature and the rate and the success of embryonic development. Journal of the Marine Biological Association of the UK 54:745-762.

Wenner AM. 1972. Sex Ratio as a Function of Size in Marine Crustacea. The American Naturalist 106(949):321-350.

Wilber DH. 1989. The influence of sexual selection and predation on mating and postcopulatory guarding behaviour of stone crabs (Xanthidae, *Menippe*). Behavoural ecology and sociobiology 24:445-451.

Zimmer M. 2001. Why do male terrestrial isopods (Isopoda: Oniscidea) not guard females? Animal Behaviour 62(4):815-821.